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SOIL SCIENCE

Contents for November, 1920

THE DETERMINATION OF NITRITES AND NITRATES IN PLANT TISSUE[1]

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INTRODUCTION

On account of the complex nature of plant juices the determination of the nitrate nitrogen therein is beset with many possibilities of error. In most of the work done upon the nitrate content of plants the Schloesing method and its modifications have been used. Some investigators have criticized this method and have recommended other methods for the purpose. Since the determination of nitrates in plants proved of great importance in the solution of a problem under investigation, it seemed necessary to make a careful study of certain methods for nitrates in order to determine their applicability to plant tissue.

Without attempting a complete discussion of the literature, the methods employed for nitrates in plant tissue, or in analogous substances will be briefly considered.

In studies of the nitrate content of plant tissue Schulze (11), Nedokvochayev (8), Woo (13) and others used various modifications of Schloesing's (12, p 456) method. This method is based upon the measurement of nitric oxide gas which is liberated when nitric acid is heated with ferrous chloride and hydrochloric acid. Krog and Sebelien (7) claim that the Schloesing method gives low results in the presence of carbohydrates and other organic substances. They obtained much more satisfactory results with the "nitron" method. In this work Krog and Sebelien used both water and alcohol (2 to 1) in making extracts of green plants. The "nitron" method devised by Busch (3) is based upon the formation of an insoluble compound by the interaction of nitron (diphenyl-endo-anilo-hydro-triazole) and nitric acid.

Caron (4) obtained good results in the determination of nitrates in urine by the colorimetric method. He determined the intensity of color produced when a nitrate solution is treated with diphenylamine and sulfuric acid.

[1] Part I of thesis submitted to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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This opportunity is taken to express to Professors Fred and Hart, due appreciation for their suggestions and criticisms.

Another method for the determination of nitrates, known as Devarda's method, is based upon the reduction of nitric acid to ammonia by means of Devarda's alloy (50 parts copper, 45 parts aluminum and 5 parts zinc) in alkaline solution (12, p. 454). This method somewhat modified in detail is recommended by Allen (1), and others for nitrate nitrogen in soils and by Davisson (5) for nitrates in "soil and physiological extracts."

In the Ulsch-Street method (2) nitric acid is reduced with iron and dilute sulfuric acid and the ammonia obtained thereby distilled from magnesia.

Zeller (14) proposed a method for determining nitrates and nitrites in the presence of much organic matter. For nitrates the Ulsch-Street method was used, while nitrites were determined by measuring the amount of ammonia decomposed by nitrous acid according to the following reaction:

$$NH_3 + HNO_2 = N_2 + 2H_2O$$

In the determination of the latter he used a known quantity of ammonium chloride and evaporated the unknown solution to a small volume. The solution was then diluted and distilled from magnesia into standard acid. The nitrite was calculated by loss of ammonia. He obtained good results with peptones, soil and plant decoctions.

EXPERIMENTAL

I. Nitrates

The colorimetric method of Caron proved unsatisfactory, as the blue color obtained by treating plant extracts with diphenylamine faded so rapidly that accurate results were impossible. The determination of nitrates in plants by finding the difference between the nitrogen obtained by the Kjeldahl method modified to include nitrate and the Kjeldahl-Gunning-Arnold method also was unsatisfactory, since appreciable amounts of nitrate were apparently reduced without zinc and salicylic acid.

The "nitron" method was next tried. The results are shown in table 1. Many substances give somewhat insoluble compounds with nitron and therefore the method must be used with caution. Among the substances likely to be present in plants that will give high results are nitrites and oxalates. While the rather meager data given above show fair results, the unexpectedly high nitrate content made it advisable to test the accuracy of the method further. On account of the variety of substances which may be present in plants that may affect the accuracy of this method, it was decided to compare it with another method based on a different principle rather than to attempt to eliminate troublesome compounds which may be present.

Consequently, the Devarda method was studied. Allen (1) claimed that this method was accurate for soils when 0.1 *N* sodium hydroxide was used in reduction instead of the more concentrated alkali originally recommended. He boiled the solution for $\frac{1}{2}$ hour before addition of alkali in order to get rid of

the "albuminoid" ammonia. Davisson showed that boiling ½ hour did not get rid of all the ammonia and he suggested previous precipitation with Stutzer's reagent to get rid of "protein like substances." However, this would not free the solution from certain amino acids (arginine and cystin), or bases (e.g. guanidine) which may be present in both soils and plants, and which would cause an error in determination by this method. A simpler and better method might be to run a control without alloy on each extract. The possible difficulties here might be:

1. That the compounds in plants yielding ammonia on boiling with sodium hydroxide may be very sensitive to a change in concentration of alkali which would be caused by the reaction of the alkali with the alloy.

2. That the alloy might act catalytically to break down some compounds which would yield ammonia.

The possibility of error from these sources was accordingly studied and the following experiments undertaken. Two proteins, lactalbumin and arachin,

TABLE 1

Nitron method for nitrates

SUBSTANCE	MODIFIED KJELDAHL METHOD	NITRON METHOD
	Nitrate nitrogen	Nitrate nitrogen
	mgm.	*mgm.*
Pure $NaNO_3$ solution	3.68	3.77
Pure $NaNO_3$ solution + nitrate-free plant extract	3.68	3.88
Plant extract containing nitrate + 7.35 mgm. N from $NaNO_3$		9.46
Nitrate nitrogen in plant extract by difference		1.90
Nitrate nitrogen extract by direct determination		1.75

both rich in arginine, were hydrolyzed by boiling for 48 hours in 20 per cent hydrochloric acid under a reflux condenser. They were allowed to cool, partially neutralized with sodium hydroxide and then made alkaline with sodium carbonate. The humin was then filtered off. The alkaline solutions were next aerated 8 hours to get rid of ammonia. Amino acids thus obtained in amounts corresponding to 0.2 gm. of protein were treated as shown in table 2.

Taken as a whole these results show that no appreciable error is caused by the difference in concentration of alkali caused by action of alloy, nor does the presence or absence of alloy affect the amount of ammonia evolved. The amount of amino acids used equals the amount in extract from several grams of flowering soybean plants grown in sand containing nitrate.

An experiment was next undertaken to determine the effect of amino acids, asparagin, and variation in the concentration of alkali on the determination of nitrates by Devarda's method (table 3).

It is again seen that a change in concentration of alkali due to the alloy's action does not affect the accuracy of the determination, and that the presence

TABLE 2

Effect of different concentrations of alkali and of alloy on NH_3 obtained by boiling amino acids with NaOH in Devarda's method for nitrates

TREATMENT	N/28 NaOH	N
	cc.	*mgm.*
Amino acids* + 250 cc. H_2O+		
1. 2.5 gm. NaOH	2.80	1.40
2. 2.5 gm. NaOH	2.80	1.40
3. 2.5 NaOH + 0.5 gm. alloy	3.20	1.60
4. 2.5 NaOH + 0.5 gm. alloy	2.90	1.45
5. 3.5 NaOH + 0.5 gm. alloy	2.90	1.45
6. 3.5 NaOH + 0.5 gm. alloy	2.90	1.45
7. 5 gm. NaOH + 0.5 gm. alloy	3.00	1.50
8. 5 gm. NaOH + 0.5 gm. alloy	3.10	1.55
9. 5 gm. NaOH + 0.5 gm. alloy	3.50	1.75
10. 5 gm. NaOH + 1.0 gm. alloy	2.80	1.40
11 5.5 gm. NaOH + 0.5 gm. alloy	3.05	1.52
12. 5.5 gm. NaOH + 1.0 gm. alloy	3.00	1.50

* Solution obtained by hydrolyzing and aerating 0.2 gm. of lactalbumin.

TABLE 3

Effect of amino acids, asparagin and variations in the amount of alkali on the determination of nitrate in Devarda's method applied to plant extracts

Solution containing amino acids* 0.02 gm. asparagin (2 mgm. amide N), and 4.45 mgm. nitrate N from $NaNO_3$ treated as below.

TREATMENT	N/28 NaOH	N	NITRATE N	AMIDE N
	cc.	*mgm.*	*mgm.*	*mgm.*
1. 2.5 gm. NaOH	6.80	3.40‡		1.95
2. 2.5 gm. NaOH	6.75	3.38		1.95
3. 2.5 gm. NaOH + 1 gm. alloy	15.60	7.80	4.41	
4. 2.5 gm. NaOH + 1 gm. alloy	15.10	7.55	4.16	
5. 3.25 gm. NaOH + 1 gm. alloy	15.40	7.70	4.31	
6. 3.25 gm. NaOH + 1 gm. alloy	15.50	7.70	4.36	
7. 3.25 gm. NaOH + 1 gm. alloy†	15.30	7.65	4.26	
8. Pure $NaNO_3$			4.45	

* Amino acids obtained by hydrolysis of 0.2 gm. of lactalbumin and subsequent removal of ammonia.

† 2.5 gm. NaOH added before reduction. 0.75 gm. NaOH added after reduction but before distillation.

‡ Mgm. N without asparagin = 1.45.

of amino acids and asparagin does not affect the difference in ammonia distilled from the controls and from the reduced solution.

Some loss was observed on boiling prior to reduction. Why this was true was not clear except that it was more difficult to keep all the liquids boiling at the same rate in the open than in a distillation. The error came principally from the incomplete volatilization of "nitrogen from sodium hydroxide" present in the controls (no. 5 and 6). The figures show that nearly half the "nitrogen from sodium hydroxide" was recovered in the distillation (table 4). A later experiment showed that better results were obtained from boiling for

TABLE 4

Effect of previous concentration of solution and boiling with NaOH in the open on the determination of nitrate in Devarda's method

4.9 mgm. nitrate N + 2.5 gm. NaOH + 0.02 gm. asparagin (2 mgm. amide N) + amino acids from arachin + 250 cc. H_2O treated as shown below.

	TREATMENT	N/28 NaOH	NITRATE N
		cc.	*mgm.*
1.	Control for 3 and 4	7.8	
2.		7.7	
3.	1 gm. alloy	17.3	4.85
4.		17.1	4.70
5.	Control for 7 and 8	1.7	
6.		1.4	
7.	Same as 3 and 4 except boiled in open ½ hour before reduction	9.7	4.05
8.		9.5	3.95
9.	Same as 3 and 4 except first evaporated from 20 to 2 cc. on asbestos sheet on hot plate before reduction		2.80
10.			2.70
11.	Same as 3 and 4 except no nitrate	7.8	
12.		7.6	

1 hour. However, the previous boiling when controls are run is unnecessary and tends to decrease rather than increase the accuracy of the method. Evaporation to a low volume on a hot plate causes a loss in nitrate. A later experiment shows that the solution can be evaporated on the water bath wihout loss of nitrate. The presence of the alloy in a non-nitrate solution gives the same result as the same solution containing nitrate but not reduced by the alloy.

The effect of the aldehyde group of glucose (which is usually present in plants) on the reduction of nitrates during this determination also was studied. The results are given in table 5.

It is seen that the presence of the sugar does not affect the accuracy of the method. The loss on boiling with NaOH for 1 hour is so slight that no con-

clusions can be drawn except that apparently previous boiling does not increase the accuracy of the method. The evaporation of the neutral solution to small volume on the water bath did not apparently affect the nitrate-nitrogen results. This last experiment was made because evaporation is necessary in the determination of nitrite.

TABLE 5

Effect of glucose on the determination of nitrate in Devarda's method

Solution containing 4.9 mgm. nitrate N and 0.1 gm. arachin (hydrolyzed and aerated) + 0.01 gm. asparagin + 0.25 gm. glucose in 250 cc. H_2O treated as shown below.

TREATMENT	N/28 NaOH	NITRATE N
	cc.	*mgm.*
1. 2. 2.5 gm. NaOH + 1 gm. alloy	13.25 13.15	4.70 4.68
3. 4. Control for 1 and 2	3.80 3.80	
5. 6. Same as 1 and 2 except boiled in open 1 hour before reduction	9.40 9.00	4.28 4.48
7. 8. Control for 5 and 6	0.50 0.40	
9. 10. Same as 1 and 2 except before reduction and addition of alkali evaporated from 25 to 3 cc. on H_2O bath	11.40 11.00	4.85 4.65
11. 12. Control for 9 and 10	1.65 1.70	

TABLE 6

Comparison of nitron and Devarda (reduction) methods for nitrates in plants

PLANT EXTRACT NUMBER	NITRATE N IN DRY MATTER		
	Devarda	Nitron	Difference
	per cent	*per cent*	*per cent*
2	0.330	0.418	0.088
5	0.375	0.440	0.065
7	0.389	0.436	0.047
8	0.200	0.244	0.044

It is seen from the above that nitrate nitrogen in plants can be quite accurately determined by the use of Devarda's alloy. This method is not applicable in the presence of nitrites. The procedure is similar to that outlined in the third paragraph of page 341. When nitrates only are being determined it is preferable to remove the soluble protein by coagulation by heat prior to the determination. This eliminates frothing and the necessity of using paraffin.

A comparison of the nitrate content of plants as shown by the nitron method and the Devarda method is found in table 6. The former method gives consistently higher results. Apparently there are present in plants compounds which cause an appreciable error by this method.

As already mentioned the Schloesing method, variously modified, for nitrates has been used by most workers in their studies on the nitrate content of plants. This method is more complicated and requires more manipulation than the Devarda method. However, on account of its wide use for the determination of nitrates in plant tissue comparative studies were made of the methods mentioned. The Devarda method modified as already described, was used in comparison with the modified Schloesing method as described by Treadwell and Hall (12, p. 456) with the further modifications suggested by Koninck (6) and Koch (13).

TABLE 7

Comparison of Schloesing's and Devarda's methods for nitrate in plant tissue

	DEVARDA'S	SCHLOESING'S
Pure $NaNO_3$ (mgm. N)	13.95	13.81
	2.79	2.71
Nodule extract	None	None
Plant extract (Nitric N, per cent of dry matter)	0.260	0.245
	0.200	0.178
	0.288	0.276
Plant juice (mgm. in 100 cc.)	30.0	23.7
	90.0	86.1

The first modification was a mechanical one whereby a mercury seal was substituted for a pinchcock for the tube leading to the gas burette. The Koch modification consisted in measuring the evolved gas absorbed by alkaline potassium permanganate instead of the total gas evolved.

Comparative determinations by this method and by the Devarda method are shown in table 7. In every case but one the results agree almost as closely as duplicate determinations by the same method. In the Schloesing method a small amount of gas was invariably found after absorption.

II. Nitrites and nitrates in the presence of one another

No attention was given in the above studies to the question of nitrites. Devarda's method would include both nitrites and nitrates, while with the nitron method according to Treadwell and Hall (12, p. 451) nitrites cause high but not quantitatively high results.

Zeller's method for nitrates and nitrites has already been mentioned. As stated, he claimed this method to be accurate for soils and plant decoctions. He does not describe the preparation of these decoctions but gives figures which indicate very accurate results. When small amounts of nitrite are present a large percentage of error will probably be found in plant tissue, especially for seedlings, since amino acids are always present which are much more reactive with nitrous acid than with ammonia. Alpha amino nitrogen reacts quantitatively with nitrous acid on shaking in acid solution for five minutes at room temperature, whereby from 1 to 1½ hours are required for the complete reaction with ammonia under the same conditions (10). In 17-day-old soybean plants 0.65 per cent α amino nitrogen was found. It is possible that a considerable amount of nitrite could be present in such cases with no loss whatever of ammonia by Zeller's method.

This error, however, could probably be avoided by the following procedure. Treat the unknown solution with ammonium chloride solution, make up to a definite volume and divide into two exactly equal portions or take similar aliquots. Determine the total nitrogen in one part immediately, and in the other after evaporating to a low volume on the water bath. Half the difference in nitrogen = nitrous nitrogen.

A simpler and shorter method which might be used for determining both nitrite and nitrate is as follows. With one aliquot of the unknown solution determine nitrate plus nitrite by Devarda's method as already outlined. Treat another aliquot with an excess of some amino compound which reacts readily with nitrous acid and which does not lose ammonia on boiling with sodium hydroxide. Heat on the water bath for a definite time. The nitrous acid reacts with the amino acid forming elemental nitrogen. Dilute to 250 cc. and determine nitrate by Devarda's method. The difference between the first and the second determination gives the nitrite nitrogen.

This method was tried and was found to give good results in the presence of nitrate and a cold-water extract of 14-day-old etiolated soybean seedlings. The amino compound fitting the above description which happened to be available was aspartic acid and this accordingly was used.

Merck's sodium nitrite, made up to equal approximately 6 mgm. of nitrite nitrogen, was used. This was found by reduction with Devarda's alloy to contain 5.57 mgm. of reducible nitrogen of which 5.2 mgm. was lost on heating on the water bath for 1 hour in initial solution of 20 cc. (evaporated to 5 cc.) with 0.15 gm. aspartic acid. No more nitrogen was lost on heating with 0.3 gm. aspartic acid to a lower volume. The plant extract was prepared by shaking vigorously by hand 8 gm. of finely ground seedlings with 100 cc. of cold water for 5 minutes, allowing to stand for about 20 minutes and again shaking for 5 minutes. This extract was then squeezed through a cloth. No attempt was made to remove soluble protein by heat or acid. The results are given in table 8. It is seen from the table that fairly accurate results are obtained. It is noted from the last pair of figures that nearly half the nitrite is lost through

the evaporation of 5.2 mgm. of nitrite nitrogen with 0.5 gm. of tissue extract without the addition of aspartic acid. This shows the fallacy of attempting to use the procedure proposed by Zeller for nitrite in plant tissue.

One per cent aqueous sodium alizarin sulfonate was used as an indicator in all this work and was found to be very satisfactory in titrating small amounts of ammonia. With the amounts employed a distinct change in color was noted at the end point with one drop of 0.0357 *N* alkali.

The question of possible losses of nitrites during extraction of the tissue, and the means by which such losses (if they exist) may be minimized or eliminated is still open for investigation.

TABLE 8

Results of test on method for nitrite and nitrate

5.2 mgm. Nitrite N + 3.8 mgm. Nitrate N + 0.5 gm. seedlings extracted + 0.15 gm. aspartic acid treated as indicated.

TREATMENT	TOTAL N/28 NaOH	NITRATE + NITRITE	NITRATE N	NITRITE N
	cc.	*mgm.*	*mgm.*	*mgm.*
1. Heated on water for 1 hour, reduced and distilled	21.4	3.5	3.5	
2. Heated on water for 1 hour, reduced and distilled	21.7	3.8	3.8	
3. Control, no alloy	18.1			
4. Control, no alloy	17.7			
5. Reduced and distilled, no previous heat	30.9	13.5		4.85
6. Reduced and distilled, no previous heat	31.0	13.6		4.90
7. Control for 5 and 6	17.4			
8. Same as 1 and 2 except no aspartic acid added	26.7	9.3		2.75
9. Same as 1 and 2 except no aspartic acid added	26.9	9.5		2.85
HNO_3 by reduction			3.8	
HNO_2 by reduction				5.17

Procedure. Dilute two equal portions of a cold-water extract of plant tissue to 250 cc. in a Kjeldahl flask. Add a small piece of paraffin and 2.5 gm. of sodium hydroxide in concentrated solution. To one solution add 1 gm. of Devarda's alloy and use the other as a control. Attach to a distilling apparatus at once. Heat under a low flame for 1 hour or until action has ceased and then distill over exactly 150 cc. Care should be taken that the determination and the control be distilled at the same rate. Titrate, using 0.0357 *N* alkali. The difference gives the nitrate plus nitrite nitrogen. The flask is attached to a distilling head containing two way bent tubes.

Treat a similar portion of cold water extract in a volume of about 25 cc. with about 0.15 gm. of aspartic acid or more depending upon the amount of nitrite present. The mixture is heated on the water bath for an hour. It is then divided into two equal portions, reduced and distilled according to the

Devarda method as given above. The difference between the first and second distillations represents the nitrite nitrogen.

SUMMARY

1. The Caron colorimetric method for nitrates in urine is not applicable to the determination of nitrates in plant tissue.

2. The determination of nitrates in plants by finding the difference between the Kjeldahl-Gunning-Arnold method and the Kjeldahl method modified to include nitrates is unsatisfactory.

3. The "nitron" method gave slightly high results with the tissue studied. In view of the substances occurring in plants which may cause error in this method it is not dependable for the determination of nitrates in plant tissue.

4. Both the Devarda and Schloesing methods with proper modifications may be applied in the determination of nitrates in plants with fair accuracy.

5. The method proposed by Zeller for the determination of nitrites and nitrates in the presence of one another is not applicable to plant tissue.

6. A procedure is suggested which gives satisfactory results for the determination of nitrites and nitrates in plant extract.

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THE RELATION OF NITRATES TO NODULE PRODUCTION[1]

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INTRODUCTION

When inoculated soybeans are grown in pure quartz sand, to which has been added an abundance of the essential plant-food elements except nitrogen, the bacteria will assimilate sufficient nitrogen to meet the normal demands of the plant. On the other hand, the soybeans will thrive equally as well without bacteria, provided sufficient combined nitrogen is supplied in available form; for example, nitrates. For the farmer it is important to know what proportion of the nitrogen of the legumes is obtained from soil and what portion is obtained from the air by means of the bacteria. The relation that exists under ordinary field conditions has been the subject of considerable study and discussion for some time, and any contribution to our knowledge of the factors governing nitrogen fixation will aid in its ultimate solution.

Many investigators have shown that certain salts inhibit, and in sufficient quantities entirely prevent, nodule formation on legume plants. Why this is true is not known and although several explanations have been offered none of these is based upon satisfactory experimental data. The purpose of this paper is to attempt to offer an explanation of the deleterious effects of large amounts of nitrates on nodule formation. While an exhaustive review of the literature is unnecessary some of the important papers will be discussed.

Effect of nitrates on legume bacteria

Hiltner (6) found that nitrates inhibited nodule formation and that the inhibiting effect of a given concentration of nitrates was much greater in solution than in soil. Prucha (15) reported that nitrates inhibited nodule formation in the Canada field pea. Wilson (19) studied the effect of various salts on nodule formation. He showed that nitrates and sulfates retarded nodule formation, while chlorides and phosphates did not. He also found

[1] Part II of thesis submitted to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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This opportunity is taken to express to Professors Fred and Hart, due appreciation for their suggestions and criticisms.

that the inhibition was local in character and when sufficient nitrate was present in soil to prevent nodule formation, the vitality of the legume organism present *in the soil* was not weakened. Fred and Graul (3) found that nitrates and ammonium salts markedly inhibited the development of nodules on vetch, alfalfa and soybeans. Mazé (10) believed that the retarding effect of nitrates was due to two causes; first, that the bacteria found sufficient nutrition outside the plant and did not enter it; and, second, that nitrates reacted with the sugar in plants and thus prevented the bacteria from obtaining sufficient sugar for their development. It has also been suggested that the immunity of the plant is strengthened by the presence of nitrogen in the form of nitrates so that the bacteria do not enter it. However, the data presented in support of any of these theories are far from convincing. Furthermore, none of them explain why certain non-nitrogenous salts, e.g. sulfates, also prevent nodule formation. It is known, that the nodules diminish in size and number with the amount of nitrate until, with sufficient nitrate present, no nodules are found. If the first theory of Mazé is correct there should be no diminution in size; instead there should be either well developed nodules or none at all. It is noted that nodules develop on plants before the reserve food of the seed is exhausted. This is out of harmony with the last-named theory. The second theory of Mazé will be considered at a later time.

Laurent (9) stated that sodium or potassium nitrate in pea or lupine decoctions in a ratio of 1 to 500 or 1 to 1000 prevented growth of legume bacteria. He stated that the nitrate alone, or the decoction alone, has no such effect. He, therefore, believed that the nitrate reacts with some compound in the plant and forms thereby a substance toxic to bacteria.

Wilson (19) has shown that when soybean bacteria are placed in a soil containing sufficient nitrate to prevent plants growing therein from forming nodules, the vitality of these organisms, as shown by their infecting power, was apparently not weakened. Hills (5) confirmed Wilson's results by using alfalfa bacteria and mannite agar slopes instead of soil. He employed a nitrate concentration up to 100 mgm. of nitrate nitrogen per 100 gm. of media. While these data show that the organism is not destroyed by relatively large amounts of nitrate, they do not prove that growth and reproduction are not hindered to a marked degree. In fact, Hills (5) showed in his studies on the effect of nitrates upon growth and reproduction of *Rhizobium leguminosarum* in soil, that while small amounts of nitrate greatly stimulated reproduction, higher concentrations produced a toxic effect, and reproduction was diminished to less than 1 per cent of the normal in untreated soil.

A fact that must be considered in connection with such studies is that the bacteria of the nodules live within, and obtain their nutriment from the plant itself, and not from the soil or its solution. As regards mineral nutrients absorbed from the soil the concentration in plant sap and in soil solution are far from equal. McCool and Millar (11) showed by osmotic-pressure determinations that roughly the concentration of plant sap increased with the

concentration of the soil solution, but the increase was not proportional. Hoagland (7) recently showed by conductivity measurements, that while the concentration of the sap varied considerably with that of the soil, the concentration of the former was 5 to 50 times greater than that of the latter. The data recorded herein (most of which were obtained before Hoagland's paper was published) are in harmony with these results.

Since Fred and Davenport (4) have shown that the different strains of *Rhizobiun leguminosarum* are sensitive to the reaction, the possibility is suggested that the toxic action of nitrates may be due to their causing a change in the hydrogen-ion concentration of the sap. While apparently no studies have been made upon the effect of nitrates on reaction, the work of Hoagland (7), Truog and Meacham (18), and Clevenger (1), show that the reaction of the plant sap is only slightly affected by the composition or reaction of the soil solution, since the reaction of the former is governed apparently by a definite buffer system.

Nitrates in plants

Schulze (16) found no nitrate nitrogen in plants grown in soil entirely free of nitrates, but nitrates were present in considerable amounts when an external supply was provided. Nedokvochayev (12) found that the nitrate content of plants increased with the nitrate content of soil although not proportionally. Woo (20) found large amounts of nitrate in *Amaranthus retroflexus* (pigweed). The amount varied at different stages of growth and in different parts of the plant.

EXPERIMENTAL

From the above discussion it is seen that while the composition of the soil solution may modify to some extent, it does not govern entirely, the composition of the plant sap. Therefore, with the object in view of trying to explain why nitrates inhibit nodule formation, comparative studies of the sap from plants treated differently were undertaken. Determinations were made of the different forms of nitrogen (nitrate, amino, amide and basic), of sugar, and of the hydrogen-ion concentration.

Since the data obtained for the various non-nitrate forms of nitrogen threw no light on this problem, they are not considered in this paper.

In order to study the effect of nitrates on nodule formation, Ito San soybeans were grown in 2-gallon jars containing 11 kilos of air-dry sand. The sand was held at 14 per cent moisture throughout the experiment. One week after planting, half of the jars were inoculated with a pure culture of *Rhizobium leguminosarum*, while the other half were treated with 1 gm. of calcium nitrate per jar each week. Every week the following nutrient solution was added to all jars: 10 cc. of 1 per cent disodium phosphate, 10 cc. of 1 per cent magnesium sulfate, 10 cc. of 2 per cent potassium sulfate and 1 cc. of 0.1 per cent ferric chloride. After 14 days the inoculated plants contained an abun-

dance of nodules, while the uninoculated plants remained entirely free from nodules. The plants were harvested when 24, 38 and 44 days old. The plants were in full bloom at the second harvest and at the third harvest young pods were just beginning to appear. The third harvest was made earlier than was expected on account of the threatened ravages of parasites. All plants were in good condition at the time of harvest. The data of the uninoculated plants are given in table 1.

Methods

The modified Devarda and modified Schloesing methods for nitrates were used. These methods are discussed in detail in part I of this paper.

Inoculated plants gave faint qualitative tests for nitrates but the amount was not measurable with the tissue available. The plants grown in sand receiving nitrates contained a large percentage of nitrate nitrogen—the fresh green plants contained from 7 to 40 times the percentage of nitrate nitrogen that had been added to the soil (table 1).

TABLE 1

Nitrate nitrogen in different parts of soybean plants; uninoculated

Greenhouse sand cultures

PART OF PLANT	AGE OF PLANT	NITRATE N ADDED TO 100 GM. OF SOIL	NITRATE N IN AIR-DRY PLANTS	NITRATE N IN 100 GM. OF FRESH GREEN PLANTS
	days	*mgm.*	*per cent*	*mgm.*
Tops	24	4.5	0.875	175
	24	4.5	0.815	163
	38	9.0	0.359	72
	38	9.0	0.458	92
	44	10.5	0.440	88
	44	10.5	0.375	75
Roots	44	10.5	0.428	86
	44	10.5	0.472	95

Occurrence of nitrates in plants grown in the field

Mammoth yellow soybeans inoculated with a pure culture of bacteria were grown on a farm near Madison. The soil was quite fertile and had received liberal treatments of farm manure for years, as well as occasional applications of limestone and floats. Perhaps on account of the richness of the soil the nodules were not very large compared with those grown in sand, although they were found in considerable numbers in all plants except in local areas throughout the field. On these areas, usually a few feet in diameter, the plants were found free of nodules, but equally vigorous and somewhat richer in nitrogen. These areas may have represented former manure piles, although no definite information could be obtained on this point. Uninoculated and inoculated plants were collected at the flowering stage and the leaves, stalks and roots

were analyzed (table 2). The leaves and stalks show very decided differences in nitrate content. The difference in the nitrate content of the roots was not so marked, but in both cases the concentration was relatively high. The data for tops and stalks show that at the earlier stages of growth the variation was possibly much greater. As further evidence that such was the case, the work of Stewart (17) shows that the nitrate content of soil is richer in the early part of the growing season than later, and McCool and Millar (11) show that roots are more sensitive to change in composition of nutrient solution than are the tops. The very low nitrate content of the nodules compared with the roots is interesting. The use of nitrate by the bacteria probably accounts for this decrease.

TABLE 2

Nitrates in inoculated soybean plants, with and without nodules, grown in the same field

Samples taken from a field near Madison

PART OF PLANT	STAGE OF GROWTH	OCCURRENCE OF NODULES	NITRATE N IN AIR-DRY PLANTS	NITRATE N IN 100 GM. OF FRESH GREEN PLANTS
			per cent	*mgm.*
Leaves	Flowering	Present	0.063	12.6
	Flowering	Present	0.075	15.0
	Flowering	Absent	0.125	25.0
	Flowering	Absent	0.123	24.0
Stalks	Flowering	Present	0.075	15.0
	Flowering	Present	0.056	11.2
	Flowering	Absent	0.175	35.0
	Flowering	Absent	0.175	35.0
Roots	Flowering	Present	0.088	17.6
	Flowering	Present	0.085	17.0
	Flowering	Absent	0.100	20.0
	Flowering	Absent	0.095	19.0
Nodules	Flowering		0.005	1.0

Occurrence of nitrates and sugar in plants grown in varying concentrations of nitrates

In order to determine the concentration of nitrate in soil and plant juice at which nodules failed to grow, plants were grown in sand containing different concentrations of nitrate. Two-gallon jars containing 11 kilos of air-dried sand and 10 gm. of calcium carbonate each were used and enough water added to bring the content up to 15 per cent. The desired quantity of sodium nitrate was added in the water. For each concentration of nitrate 8 jars were planted with Medium Early Green soybeans. The plants were removed from 4 jars for each duplicate determination. All jars were inoculated when the plants were 12 days old and again a week later. The nutrient solution was added weekly,

beginning 14 days after the soybeans were planted. The same kind and amounts of non-nitrogenous nutrients that were used in the former greenhouse experiment also were added in these tests.

Nodules were observed first in the control plants when 22 days old, or 10 days after the first inoculation. The plants were harvested when 31 days old. Immediately after harvesting the roots were washed free from sand in running water and then shaken as free as possible from water. The roots were then cut off from the tops and chopped into fine pieces. This finely cut tissue was then thoroughly macerated in an agate mortar and the juice squeezed through canvas into a test tube. The test tube was immediately corked and placed in a freezing mixture of ice and salt. About 15 minutes elapsed between the

TABLE 3

Effect of nitrates on reducing sugar and nitrate content of plant sap (afternoon harvest)

Plants grown in sand

CONDITION OF PLANTS AT HARVEST	NUMBER AND SIZE OF NODULES	NITRATE N ADDED TO 100 GM. OF SAND	NITRATE N IN 100 CC. OF PLANT JUICE		REDUCING SUGAR IN PLANT JUICE*
			Tops	Roots	Roots
		mgm.	*mgm.*	*mgm.*	*mgm.*
Thrifty	Large; abundant	0	30.0	4.0	342
Thrifty, smaller than above	Small, few	2.5	93.7	47.0	421
	None	5.0	93.7	56.1	187
	None	10.0	100.0	72.2	168
No germination		20.0			

* Calculated as mgm. of dextrose per 100 cc. of juice.

harvest and placing the extracted juice of tops and roots in the freezing mixture. The juice was kept cold until the analyses were made. The analyses were completed within 48 hours after harvest. The results are given in table 3.

The data are in conformity with those made on the dry matter. The nitrate content of the juice increases with the nitrate content of the soil, although the increase is not nearly so great proportionally.

The reducing sugar content decreases with the increase in nitrate, although a considerable amount is present even with the highest concentration of nitrate.

These plants were harvested in the afternoon when the sugar content probably approached the maximum. In order to determine the sugar and nitrate content of the root sap in the early morning another set of plants was grown.

For this second experiment new sand was obtained for the controls while the same sand which originally contained 5, 10 and 20 mgm. of nitrate nitrogen, respectively, used in the previous experiment, was again used.

The plants were harvested when 26 days old and had attained approximately the same stage of growth as those in the previous experiment. The control plants were somewhat larger than the plants grown in the lowest concentration of nitrate. Ten milligrams of nitrate depressed growth to a greater extent while the seed again failed to germinate in the sand containing 20 mgm. of nitrate nitrogen per 100 gm. The harvests and extractions were made from 6 to 7 a.m.

The control plants contained large nodules while the plants from the first concentration contained very small nodules, barely visible to the naked eye. The plants grown in the higher concentration were entirely free from them. The results are given in table 4.

The reducing sugar content was lower and the nitrate content higher in plants collected in the morning than in those collected in the afternoon. The plants not being grown at the same time, of course, are not exactly comparable.

TABLE 4

Effect of nitrates in sand on reducing sugar and nitrate content of plant sap

Early morning harvest

HEIGHT OF PLANTS	GERMINATION	NITRATE N ADDED TO 100 GM. OF SAND	IN 100 CC. OF ROOT JUICE	
			Nitrate N	Reducing sugar
inches		*mgm.*	*mgm.*	*mgm.*
7½	Good	0	17.5	220.0
6	Good	5	87.5	107.4
5	Less than 50 per cent	10	120.0	75.8
0	None	20		

The accumulation of nitrates in darkness

The figures given in tables 3 and 4 indicate that nitrates accumulate in plants during the night. It is, therefore, interesting to know to what extent they may accumulate during longer periods of inhibited photosynthesis. The following studies, therefore, were made.

Jars containing 37-day-old plants grown in sand to which 10.5 mgm. of nitrate nitrogen from calcium nitrate had been added were removed to a dark cellar. Three days later another lot of the same plants was placed in the cellar. Jars were kept also in the light for controls.

All plants were harvested when 44 days old and analyzed for nitrate. The results are given in table 5.

It is seen that there was a decrease of nitrate in the tops but an increase in the roots. This may possibly be explained on the basis of decreased transpiration and the consequent decreased upward movement in the cool, damp cellar. The residual sugar in the leaves used up the nitrates faster than it was moved upward, although not as fast as it was absorbed by the roots. As further evidence that such an explanation may be the correct one, it was

found that when 38-day-old plants were placed in a warm, dry, dark closet for 3 days the percentage of nitrate in the tops was greatly increased.

From the data presented it appears that in the other experiments the nitrate content of roots when harvested probably was as low as or lower than at any other time of the day, with the exception of the results reported in table 4.

TABLE 5

Effect of darkness on the accumulation on nitrates in plants

Sand cultures

AGE OF PLANTS IN LIGHT	TIME KEPT IN DARKNESS	NITRATE N ADDED PER 100 GM. OF SAND	NITRATE CONTENT IN 100 GM. OF SAP	
			Tops	Roots
days	*days*	*mgm.*	*mgm.*	*mgm.*
44	0	10.5	110	118
44	0	10.5	83	107
40	4	10.5	77	140
37	7	10.5	61	145

Nitrate content of roots from the same plant when divided parts are placed in nitrate and non-nitrate solutions

Wilson (19) reported that when the roots of a soybean plant were divided and one portion placed in an inoculated solution containing no nitrate and the other portion in an inoculated solution containing nitrate, the roots in the non-nitrate solution developed nodules while those in the nitrate solution developed none. He concludes that the inhibiting factor is local in character. If, as the data reported thus far indicate, there is a connection between the nitrate content of the plants, and the failure of the plants to form nodules, the nitrate content of two root portions should differ. Accordingly, experiments were made to determine whether or not such a variation existed.

The amount of nitrate in the divided root portions was determined as follows. In two ordinary glass tumblers was placed 250 cc. of a nutrient solution without nitrogen. To one of these tumblers was added sufficient calcium nitrate to give a 0.05 per cent concentration of the salt (20 mgm. of nitrate nitrogen). Roots from 6 inoculated soybean plants (40 days old) were divided as evenly as possible and one portion was placed in each tumbler. The water lost was renewed daily. Roots from 24 plants were harvested after 3 days for duplicate determinations. The roots from each portion were cut off and thoroughly washed, and after shaking as free as possible from adhering water the roots were blotted between filter paper. They were then cut up fine, macerated in a mortar and the juice squeezed through canvas. The nitrate determinations were completed within 3 hours after harvesting.

To another set of plants a second addition of 20 mgm. of nitrate nitrogen as calcium nitrate was added to the nutrient solution after the fifth day.

These plants were allowed to remain in the solution for a total of 8 days. The results with the two sets of plants are given in table 6.

The non-nitrate solution gave a negative qualitative test for nitrate at the end of the 8-day period.

These results show a decided difference in the nitrate content of the roots in the two portions. This is in harmony with the results of Nedokvochayev (12) and of Kraus and Kraybill (8) who have shown that different parts of the same plant organs may differ in nitrate content.

TABLE 6

Nitrates in different portions of roots of the same plants (40 days old) when different portions are placed in nutrient solutions with and without nitrates

DAYS IN SOLUTION	NITRATE QUALITATIVE	NITRATE	
		In 100 cc. of nutrient solution	In 100 cc. of juice.
		mgm.	*mgm.*
3	Present	0	Trace†
3	Present	0	Trace†
3	Present	8.0	8.46
3	Present	8.0	8.46
8	Present	0	1.20
8	Present	0	0.90
8	Present	16.0*	10.16
8	Present	16.0*	Lost

* 8 mgm. added at beginning; 8 mgm. added after 5 days.

† Less than 1 mgm. per 100 cc. of juice.

The effect of nitrates upon the reaction of the plant juice

It has been noted earlier in this paper that several investigators have shown that the reaction of the plant sap is governed by a buffer system, and that this reaction is to a considerable degree independent of the composition and reaction of the soil solution. However, since the specific effect of nitrates on reaction had not been studied, the determinations of hydrogen-ion concentration of the juice from the same set of plants reported in table 4 were made. Plants 26 days old were harvested in the afternoon, and the determination made immediately. It was found that untreated plants had a hydrogen-ion concentration, expressed as pH, of 5.82, plants receiving 5 mgm. of nitrate nitrogen had a pH of 6.14 and plants receiving 10 mgm. a pH of 6.4.[2]

These figures show that sodium nitrate decreases the acidity (H-ion concentration) to a slight extent, but the pH in the nitrate-containing plants is even more favorable for the growth of *Rhizobium leguminosarum* than the reaction of the sap of plants containing no nitrate. Apparently, the inhibiting effect of nitrates on nodule production is not a question of reaction.

[2] These determinations were made by O. C. Bryan.

DISCUSSION

It was pointed out in the introductory part of this paper that two theories explaining why nitrates inhibit nodule production are not tenable in the light of observed facts. Experiments were made to determine the validity of the other theory, namely, the failure of the bacteria to thrive on account of the lack of sugar, since the sugar was used up in reacting with the nitrate. The data obtained showed that while the sugar content was less in the plants containing nitrate, the soluble reducing sugar did not entirely disappear from the nitrate-containing plants even in the early morning after the nocturnal period of the arrested photosynthesis.

From the studies reported herein it is seen that when soybeans are grown in soil or sand containing nitrate there is a marked accumulation of nitrates in the roots and tops of the plants and that normally, the concentration of nitrate is much greater in the plant than in the soil, or even in the soil solution. It was also observed that nitrates in the plants increased to some extent with the increase of the nitrates in the soil. Furthermore, when plants are grown in a sand very poor in nitrate, comparatively small amounts of nitrate are found in the plant.

Hills' (5) results on the effect of nitrates on the growth and reproduction of *Rhizobium leguminosarum* are given in table 7. It is noted from these results that the amounts of nitrates (2.5, 5.0 and 10 mgm. of nitrate nitrogen per 100 gm. of soil) added to sand in the greenhouse experiments reported in this paper, actually stimulated growth and reproduction, but that *the concentration of nitrate similar to that found in the plant when nodule production was inhibited, was sufficient to bring growth and reproducion of the bacteria in the soil to a virtual standstill.* The decrease in growth due to the increase in nitrate is gradual, just as in the plants there is a gradual decrease in the size of the nodules with an increase in the amount of nitrate. Likewise, the analyses of plants containing small nodules grown in a fertile field showed enough nitrates, according to the table, to have some inhibiting effect upon the growth of *Rhizobium leguminosarum.* Inoculated plants entirely free from nodules, from the same field, contained decidedly more nitrate.

It was shown that when different portions of roots from the same plant are grown in nitrate and non-nitrate solutions the nitrate content of the portion grown in nitrate was 10 times greater than the portion grown in the non-nitrate solution.

Whenever an inhibiting effect on nodule production occurred in these studies sufficient nitrate was found *in the plant* to check to a considerable degree the reproduction of *Rhizobium leguminosarum* in soil as shown in table 8. The amount of nitrate present in the plant was several times greater than the nitrate content of the soil in which the plant was grown; also there was no definite relationship between the nitrate content of the soil and that of the plant. On account of relatively rapid diffusion in the plant sap the nitrate consumed

by bacteria in the plant roots would probably be more quickly replaced than would be the case in either soil or agar. Therefore, aside from the buffer actions of the soil particles on substances toxic to organisms, a given concentration of nitrate may be more effective in inhibiting the growth of bacteria in the sap than in the soil or agar. It is obviously impossible to determine the exact effect of a specific concentration of nitrate upon legume bacteria in the

TABLE 7

Influence of calcium nitrate on Rhizobium leguminosarum in sterilized soil

Results obtained by Hills (5)

CULTURE NUMBER	TREATMENT* (NITRATE N IN 100 GM. OF DRY SOIL)	NUMBER OF ORGANISMS IN 1 GM. OF DRY SOIL				
		At the beginning	After 1 week	Relative	After 2 weeks	Relative
	mgm.			*per cent*		*per cent*
1		10,000	960,000	100	4,675,000	100
2		10,000	850,000		4,590,000	
3	2.3	10,000	3,650,000	419	6,000,000	124
4		10,000	3,940,000		5,450,000	
5	6.0	10,000	5,500,000	674	10,650,000	274
6		10,000	6,700,000		14,700,000	
7	11.5	10,000	4,000,000	414	9,350,000	195
8		10.000	3,500,000		8,670,000	
9	23.0	10,000	1,200,000	180	1,500,000	35
10		10,000	2,050,000		1,750,000	
11	35.0	10,000	865,000	106	765,000	17
12		10,000	1,050,000		800,000	
13	46.0	10,000	375,000	35	350,000	7
14		10,000	260,000		300,000	
15	69.0	10,000	35,000	4.5	25,000	0.7
16		10,000	47,000		40,000	

* Hills reported the soil treatment in terms of nitrate ($+NO_3$). These figures have been converted to the nitrogen (N) equivalent in order to harmonize with the other data.

living plant root, and while, of course, the effect of a given concentration in soil cannot be exactly comparable to the effect of the same concentration of nitrate in the plants, the data reported herein, interpreted in the light of data secured by others, offer striking evidence that the inhibiting action of nitrate upon nodule formation is at least in part due to the antiseptic action of the nitrate of the root sap upon *Rhizobium leguminosarum*. That this action is probably not due to any change in osmotic pressure is seen from Wilson's (19) results, namely, that nitrates and sulfates inhibit nodule production but phos-

phates and chlorides have an opposite effect. This would appear to indicate that the inhibiting action is of a specific nature. Also data are reported above which prove that the effect is not due to a change in reaction.

Kraus and Kraybill (8) have shown that a high nitrate content in plants accompanies a low sugar content and vice-versa. Data reported herein confirmed their results. Consequently, any evidence that nitrates *in the plant* exert a detrimental effect upon legume bacteria also may be considered as evidence that a decreased sugar supply is responsible. On the other hand, the work of Hills, previously discussed, shows that the deleterious effect of an excess of nitrates on legume bacteria takes place in soil where photosynthetic processes are not involved. However, before any definite conclusion can be drawn in this regard, it will be desirable to determine whether or not the addition of sugar to the soil counteracts the depressing action of nitrate. Likewise, although sulfates and nitrates independently exert an inhibiting effect on nodule production, it may be contended that the effect of sulfates could be due to the possible stimulation of the nitrogen metabolism of the plants and the resultant increased consumption of sugar. Further studies should also be made regarding this point.

Furthermore, it would be interesting to know the relative absorption and accumulation of the various salts by legume plants. It may be noted in this connection that Peterson (13) found a large accumulation of sulfates in plants grown in soil to which sulfates had been added. The behavior of sulfates in that respect is thus seen to be similar to that of nitrates. The effect of high concentrations of sulfates upon the growth and reproduction of legume bacteria in soil or in solution has not been studied. Pitz (14) found that 1 per cent calcium sulfate had little effect upon the total number of organisms in the soil. Fred and Hart (4) studied the effect of different sulfates upon carbon-dioxide production in soil. They found in general that small amounts produced a marked stimulation in carbon-dioxide production, and that an optimum concentration was reached, after which further sulfate additions produced a decline in the carbon-dioxide output.

Another interesting study that this work suggests is the antiseptic action of absorbed radicals on phytopathogenic organisms. The statement is often made that fertilizers give the young plant more vigor and render it more resistant to disease. To determine whether this increased immunity is at least partially due to the accumulation of certain salts absorbed by the plant from the fertilizers would make a very interesting and important subject of investigation.

SUMMARY

When soybeans are grown in soil or in sand containing nitrates there is a marked accumulation of nitrates in the plant.

The concentration of nitrate in the plant sap is much greater than in the soil or even in the soil solution.

There is an increase in the nitrate content of the roots during periods of arrested photosynthesis.

The nitrate content of sap increases to some extent with the increase of nitrate in sand, although the increase is not proportional.

Nitrates retard, and in sufficient quantities entirely prevent, nodule formation.

Nitrates have little effect upon the hydrogen-ion concentration of the plant juice.

The concentration of nitrate present in plant sap when the plants fail to produce nodules is sufficient practically to prevent growth and reproduction of soybean bacteria in soil. On the other hand, the concentration of the nitrate in the soil in which the plants were grown is so low that growth and reproduction *in soil* are stimulated.

While different buffer actions and rates of diffusion make exact comparisons between the effect of different concentrations in soil and in sap impossible, the data presented offer strong evidence that the reason for failure of nodule production in the presence of nitrates is due at least in part to the effect of the high concentration of nitrate in the sap upon the growth and reproduction of *Rhizobium leguminosarum*.

This theory is not out of harmony with Wilson's findings that the inhibiting factor was local in character.

The amount of reducing sugar in plants decreases with the increase in nitrate, but sugar was present with the highest content of nitrate used even in the early morning. Further study is needed before definite conclusions can be reached as to what extent the failure of nodule production in the presence of nitrates is due to a diminished sugar supply.

Some evidence indicates that the deleterious effect of the high nitrate concentration upon *Rhizobium leguminosarum* is at least partially of a specific nature.

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THE CAPILLARY POTENTIAL AND ITS RELATION TO SOIL-MOISTURE CONSTANTS

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Although a slight change in the structure of a given soil may appreciably change the value of the potential with constant moisture content, we may nevertheless make use of this physical character to advantage in soil-moisture studies. Buckingham (2) has outlined its significance in the study of moisture movement but recent literature is devoted largely to another point of view.

The potential may involve other factors but obviously a soil which has been allowed to approach a condition of permanent porosity by processes akin to cropping may be characterized by a capillary potential, the magnitude of which is determined by the moisture content and the concentration of dissolved substances.

Briggs (1) has given some preliminary data obtained from a series of soils by use of the "centrifugal" machine, showing that for a considerable range the moisture content ρ is a linear function of the reciprocal of the "centrifugal" force with which it is in equilibrium. If this force is proportional to the potential φ for the corresponding moisture content, the potential is evidently an hyperbolic function of the moisture content over this range, the effective radius r of the soil particles entering as a parameter. This subject has not, however, been exhaustively investigated and it is not unlikely that the function is somewhat more involved.

Whatever may be its form, it is of interest to note that a new interpretation of soil-moisture constants is made available through a consideration of the potential function. The so-called hygroscopic coefficient h, regarded as a function of the soil texture, defines an equipotential curve over the $\varphi\rho r$ surface; and, in a similar manner we may regard the Hilgard-Briggs moisture-holding capacity c, the saturation constant s, and the Briggs moisture equivalent e as specifications of particular equipotential curves. The wilting coefficient w may involve other factors such as operate to influence the *movement* of soil moisture, although it will no doubt define a curve of approximately constant potential. The same may be true of Greaves' (4) biological constants, m_a, m_n, m_{as}.

The curves of figure 1 have been drawn to illustrate the projection into the $\varphi\rho$ plane of a series of Briggs' curves for different kinds of soil. As stated, he has plotted the reciprocal of the "centrifugal" force against the moisture con-

tent and gets a series of straight lines, whereas, the curves of figure 1 would perhaps more nearly represent the potential curves. A system of concurrent straight lines were drawn and the equipotential lines e, w, and h so located as to give a system of values for the ordinates at the points of intersection with the curves of the family satisfying Briggs' system of linear equations. These curves were extended in such a way as to approximate a family of hyperbolae intersecting the vertical line c at points the ordinates of which would also satisfy these equations.

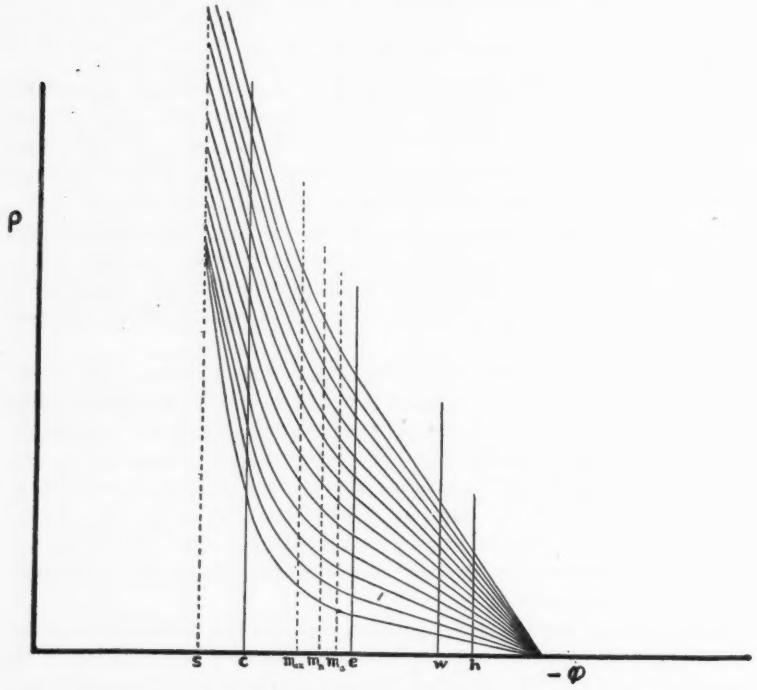

FIG. 1. A SYSTEM OF CURVES SATISFYING BRIGGS' LINEAR EQUATIONS AND CONSISTENT WITH HIS DATA RELATING THE MOISTURE CONTENT TO THE RECIPROCAL OF THE EQUILIBRIUM CENTRIFUGAL FORCE

A comparatively simple transformation of the points on these curves parallel to the φ axis is no doubt possible which would give a family of hyperbolae. It is evident, however, that the lines must remain concurrent at a finite point corresponding to the "adhesion" potential which would perhaps be the same for all soils.

A series of vertical lines have been drawn to illustrate the projection into the $\varphi\rho$ plane of the several equipotential curves indicated above. The ordinates at the various points of intersection would represent the various constants

for the series of soils represented, and it may be readily seen that a knowledge of the functional relation between the potential, the moisture content, and the effective radius of the soil particle, whether obtained empirically or by theoretical speculation, would not only be equivalent to a knowledge of the various moisture constants but also relations that may exist between them such as the Briggs system of equations (3).

Attention should be called to the fact that the function may not be single-valued. It is not difficult from a careful consideration of the surface configuration of the moisture in the soil to conceive of an abrupt change from positive to negative curvature at a given moisture content due to an abrupt uniting of the numerous individual droplets about the points of contact of the soil particles, resulting in a new surface configuration of reverse curvature. The potential would be directly determined by the curvature and two values may thus be possible for a given moisture content over a certain region. In fact, it seems quite impossible to account for a hygroscopic coefficient on any other basis.

Fortunately, a simple method of experimentally determining the potential function is available, as suggested by Buckingham (2). It is perhaps quite immaterial where the zero potential is placed and also what convention is adopted as to the algebraic sign, although it is somewhat more in accord with modern usage to define the potential as the work done by the field forces in bringing unit mass from the point in question to infinity, and in such case the heat of vaporization corresponding to the potential at s on the diagram should be added to Buckingham's potential and the negative sign should be used.

In conclusion, it may be stated that a consideration of the subject of soil moisture from the standpoint of the capillary potential gives a new interpretation of the various soil moisture constants. From experimental data already available, it is evident that the potential function may be comparatively simple. The curves which have been plotted are given as indicative of what may be expected although further experimental investigation may suggest a transformation parallel to the potential axis. They are consistent with the well known Briggs equations and with the linear character of his moisture-centrifugal force curves, although, as stated, they are to be regarded as suggestive only, and this article has been written with the hope of emphasizing the point of view rather than to attempt to specify correctly the character of the function.

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INFLUENCE OF MOISTURE ON THE BACTERIAL ACTIVITIES OF THE SOIL

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Most of the changes which take place in soil are wrought by micro-organisms. They bring about the transformations through which nitrogen passes into the soil, that is to say, the transformation from its organic compounds of the soil or its free form of the atmosphere to a form available to the growing plant. Furthermore, bacteria play an essential part in the cycles through which hydrogen, sulfur and carbon pass. They bring about the mineralization of calcium, iron, phosphorus and other inorganic elements of the plant and animal residues in the soil. Moreover, many compounds are changed from insoluble to soluble forms and thus made available to the growing plant. At times bacteria have an opposite effect and render many of these substances insoluble, thus preventing their loss from the soil through leaching. Or at times they may even compete with the higher plants for the limited supply of nutrients in the soil.

The speed with which these transformations proceed is governed to a marked extent by the water which the soil contains and it is not unlikely that the optimum moisture content of a soil for the production of maximum crops is that water content which is ideal for beneficial bacterial activity of that specific soil. Although investigators have determined the optimum moisture content for the various bacterial activities, yet none have made an attempt to obtain a general expression to cover specific bacterial activities in widely different soils. This investigation was therefore undertaken with the hope of finding some general law governing the water requirements of the various bacteria of the soil and correlating this with the requirements of higher plants.

A careful review of the literature dealing with the various phases of the subject has been made, and there is given under each division a resumé of the most important.

SOILS

The soils used in the work were collected during the fall of 1919. They represent 22 different farms, and all except three were from Cache Valley, taken within a radius of 45 miles within the basin of what used to be Lake Bonneville. They are of sedimentary origin and came from the nearby

mountains which are composed largely of quartzite and limestones. All are well supplied with phosphorus, potassium and other essential elements (49), with the exception of humus and nitrogen which in the majority of cases is low, as is characteristic of the soils of arid America. Some of the soils con-

TABLE 1

Soils used in determining moisture requirement of bacteria

SAMPLE NUMBER	TYPE OF SOIL	LOCATION	CROP	TREATMENT
1	Clay loam	J. C. Johnson Farm, North Logan	Beets—8 years	Irrigated, manured
2	Clay loam	J. C. Johnson Farm, North Logan	Beets—8 years	Irrigated, manured
3	Tight clay loam	J. C. Johnson Farm, North Logan	Alfalfa—2 years	Irrigated, manured
4	Sand loam	North Logan	Weeds	Irrigated, manured
5	Light sand loam	Jacob Swartz Farm, E. North Logan	Wheat—2 years	Dry land
6	Clay loam	Parley Armond Farm, North Logan	Wheat	Irrigated
7	Peaty loam (alkali)	R. Smith Farm, West Logan	Pasture	Irrigated
8	Silt loam	East Petersboro	Wheat, continually	Dry land
9	Black loam	Miller Bros. Farm, E. Petersboro	Wheat	Dry land
10	Very tight clay	Kidman Farm, Petersboro	Barley	Dry land, no manure
11	Silt loam	Near Mendon	Wheat—8 years	Dry land, no manure
12	Extra tight clay	Near Mendon	Barley	Dry land, no manure
13	Trenton fine loam	Sugar Spur, W. Logan (McCombs)	Beets—3 years	Irrigated, manured
14	Fine silt loam (alkali)	Johnson Farm, West Logan	Beets—2 years	Irrigated, no manure
15	Light sandy loam	Providence (Hanson Farm)	Beets—15 years	Irrigated, manured
16	Medium sand loam	Providence (Allen Farm)	Corn	Irrigated, manured
17	Sand	Providence Bench	Nothing	Dry land
18	Light mountain loam	Bothwell, Utah (Sorenson Farm)	Wheat	Dry land
19	Loose, light mountain loam	Johnson Farm, Blue Creek	Wheat—4 years	Dry land
20	Fine sand	Hansen Farm, Garland	Fallow, wheat	Dry land
21	Organic loam	Hansen Farm, Collinston	Wheat—12 years	Dry land
22	White clay loam	Poulson Farm, Collinston	Wheat—10 years	Dry land

tain as high as 50 per cent of calcium and magnesium carbonate. They are all remarkably fertile and produce excellent crops for many years without the addition of manures. Although quite similar they possess a great variation in physical properties. Most of the soils were ideally adapted both chemically and bacteriologically to support rapid bacterial action.

The type, location, crop and general treatment of the various soils are given in table 1.

These soils represent the typical farming lands of Cache Valley—dry land, irrigated, manured, and unmanured. Their treatment has been greatly varied and although we give here the crop for only a few years, yet some of them have been tilled for more than fifty years.

They range all the way from the loose sand, as represented by no. 17, to the tight clay of no. 12, as may be seen from table 2.

TABLE 2

Physical analysis of soils used in determining moisture requirement of bacteria

SAMPLE NUMBER	MEDIUM SAND	FINE SAND	COARSE SILT	MEDIUM SILT	FINE SILT	CLAY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	27.544	35.966	14.121	9.972	6.173	7.083
2	21.001	44.250	21.086	11.087	2.098	5.240
3	43.676	53.805	19.521	10.674	4.327	9.101
4	18.282	33.085	19.804	12.785	9.340	6.983
5	26.884	27.270	21.421	11.403	7.443	7.715
6	4.500	26.487	27.905	17.955	9.974	11.363
7	6.049	31.578	26.077	18.594	7.767	14.450
8	12.501	21.963	20.260	15.195	11.137	11.033
9	8.010	24.867	24.176	20.441	10.245	16.136
10	3.630	19.502	25.020	24.785	18.837	12.642
11	15.560	33.799	16.928	14.764	9.948	11.313
12	1.086	6.363	17.866	27.381	26.227	19.896
13	20.806	41.067	19.133	9.025	6.015	7.209
14	17.267	30.072	22.536	16.375	3.685	2.265
15	77.882	9.374	5.539	3.566	3.035	4.841
16	14.577	35.581	23.345	13.576	6.690	7.485
17	90.140	7.941	1.252	0.703	0.443	0.831
18	39.965	30.275	13.751	5.377	5.010	7.409
19	28.252	39.064	11.774	8.822	5.860	6.302
20	89.104	4.571	1.539	1.925	1.754	2.079
21	13.074	26.350	23.957	14.794	12.510	1.018
22	20.129	31.338	15.749	12.645	8.195	13.340

The analysis was made by means of the Yoder elutriator (53), and the different separates are those indicated by him. The medium sand ranges from 1.086 in no. 12 to 90.14 in no. 17. The fine sand varies from 4.571 in no. 20 to 53.805 in no. 3, the coarse silt from 1.252 in no. 17 to 27.905 in no. 6, medium silt from 0.703 in no. 17 to 24.785 in no. 10, fine silt from 0.443 in no. 17 to 26.227 in no. 12, and the clay from 0.831 in no. 17 to 19.896 per cent

in no. 12. Thus we have a wide variation in so far as physical composition is concerned and hence their bacterial analysis should indicate the different moisture requirements of various soils.

MOISTURE-HOLDING CAPACITY

This was determined by the method devised by Hilgard and modified by Briggs (37). Small cups 5 cm. in diameter and 1 cm. in height with the bottom made of fine screen were used. The soil was settled slightly by jarring and stroked off level with the top of the cup. The filled cups were then placed with the bottom in water, and when the soil had taken up their maximum amount of water they were allowed to drain for 30 minutes. The percentages of moisture in the soil were then determined by weighing before and after drying.

The average results for four closely agreeing determinations are given in table 3.

TABLE 3

Maximum water-holding capacity of soil

NUMBER	WATER-HOLDING CAPACITY	NUMBER	WATER-HOLDING CAPACITY
	per cent		*per cent*
17	31	10	61
20	33	12	61
15	44	2	61
18	51	4	62
13	51	3	65
5	53	1	68
11	56	7	74
19	56	8	77
6	57	14	78
9	59	21	78
16	60	22	78

This gives us a set of soils with a water-holding capacity of from 31 to 78 per cent. It is quite evident from these results that the water-holding capacity of these soils is due in the main to the quantity of clay and organic matter. Soil 17 has the lowest water-holding capacity and it also has the lowest percentage of clay. Soil 21, with the highest water-holding capacity, is next in clay content, but it possesses more organic matter than any of the other soils.

METHOD OF EXPERIMENTATION

The ammonifying, nitrifying, and nitrogen-fixing powers of the soil with the various moisture contents were determined as follows:

The ammonifying power of the soil was determined by weighing 100-gm. portions of the soil and 2 gm. of dried blood into sterile tumblers and covering

them with petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to from 10 to 100 per cent in 10 per cent increments of their water-holding capacity. The samples were incubated at 28° to 30°C. for 4 days and the ammonia determined by transferring to Kjeldahl flasks with 400 cc. of distilled water, adding 2 gm. of magnesium oxide and distilling into 0.1 *N* sulfuric acid.

The nitrifying power of the soils was determined in tumblers similar to the determination of the ammonifying power, except that they were incubated for 21 days. The moisture content was made up every 3 days to the initial percentages, and the nitric nitrogen determined as follows (18).

The contents of the beaker, together with 500 cc. of distilled water and 2 gm. of alum, were placed in quart Mason jars and agitated for 5 minutes in a shaker.

An aliquot part (100 cc.) of the supernatant liquid was pipetted off, and, together with 2 cc. of a saturated solution of sodium hydroxide, was evaporated to about one-fourth of its original volume to free it from ammonia. To this were added 50 cc. of ammonia-free water, 5 gm. of "iron-by-hydrogen," and 30 cc. of sulfuric acid (sp. gr. 1.35). If less than 40 mgm. of nitric nitrogen is to be determined, it is well to take a correspondingly smaller quantity of iron and sulfuric acid. The neck of the reduction flask was fitted with a 2-hole stopper, through which passed a 50-cc. separatory funnel and a bent tube which dipped into a vessel containing water in order to prevent mechanical loss. The acid was slowly added and allowed to stand until the rapid evolution of hydrogen was over. It was then heated to boiling for 10 minutes. The contents of the side vessel were returned to the reduction flask before the reaction was complete, thus insuring the complete reduction of any nitrates which may have been carried over with the first violent evolution of the hydrogen. The contents of the reduction flask were transferred to Kjeldahl flasks, neutralized with sodium hydroxide, and distilled into standard acid. The excess of acid was titrated back with standard alkali, lacomoid being used as an indicator. Controls were run on all the reagents including the alum used as a flocculant.

The nitrogen-fixing powers of the soil were determined by weighing 100 gm. of soil and 1.5 gm. of lactose into sterilized tumblers covered with petri dishes. Sufficient sterile distilled water was added to bring the moisture up to the required percentage. The samples were incubated for 21 days at 28° to 30°C. The moisture content was made up to the required content every other day. The tumblers and contents at the end of the incubation period were dried at 95°C., ground in a mortar and 20-gm. portions used for the determination of nitrogen by the Gunning method revised to include nitrates (21).

In the ammonifying, nitrifying and nitrogen-fixing work five or six determinations were made at each water content and the results as reported, throughout this work are the averages of a number of closely agreeing determinations.

Ammonifying powers. The influence of moisture on the ammonia found in the soil is very great. Lipman and Brown (32) found ammonification in a loam soil to increase with increased water content even up to 35 per cent of the weight of the soil. However, later they and Owen (33) found ammonification to increase as the water added increased up to a certain percentage, which varied with the physical nature of the soil, but larger quantities of water reduced the ammonia recovered. The work clearly demonstrated that the optimum moisture content for maximum ammonification is higher than it is for maximum nitrification. The quantitative difference between the two processes in the same soil was found by Sharp (44). Ammonification was most rapid with a 25 per cent moisture content and was not markedly affected by 3 per cent differences. Nitrification was at its maximum when the soil contained 19 per cent of water. When it was increased to 25 per cent the rate of nitrification was decreased 50 per cent.

When soils are held at a certain moisture content for several months and then all brought to a corresponding moisture content (20 per cent) and the ammonia determined after 4 days, the variation in moisture content affects very materially the ammonia produced, as seen from the following results obtained by Greaves and Carter (17):

MOISTURE ADDED	PER CENT OF AMMONIA PRODUCED
12.5 per cent of water	100
15.0 per cent of water	111
17.5 per cent of water	113
20.0 per cent of water	123
22.5 per cent of water	119

This increased ammonification with increased moisture content is due, according to Lipman, to the suppression of the aerobic decay bacteria and an acceleration of the anaerobic putrefactive bacteria.

Robson (41) studied the changes produced in the nitrogen compounds in the natural organic matter of soils and ammonium sulfate and horn meal in sandy loam and clay soils with varying amounts of water (6, 12 and 18 per cent in sandy soil; 8, 16 and 24 per cent in loam; and 8, 18 and 28 per cent in clay). With a low moisture content the transformations of organic nitrogen were more rapid in sandy soils than in the heavy soils, whereas with a higher moisture content there was little difference.

The work at the Utah Agricultural Experiment Station (20) demonstrated that the application of water to a soil increased its ammonifying powers, as is shown in the following:

	per cent
No water	100
15.0 inches of water	103
25.0 inches of water	97
37.5 inches of water	104

The soil which received no irrigation water in these plats was taken as producing 100 per cent of ammonia.

The results which we have obtained in the study of the ammonifying powers of the 22 soils previously described are given in table 4. The water contents of the soil were 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per cent of its water-holding capacity. The results as reported are the averages of four or more closely agreeing determinations.

TABLE 4

Ammonia formed in 100 gm. of soil maintained at various moisture contents expressed in per cent of water-holding capacity

NUMBER	KIND OF SOIL	AMMONIA FORMED									
		10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent	80 per cent	90 per cent	100 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	Clay loam	0.3	28.0	51.0	65.0	76.0	135.0	127.0	72.0	71.0	69.0
2	Clay loam	2.0	20.0	39.0	91.0	97.0	109.0	63.0	54.0	49.0	57.0
3	Tight clay loam	0.3	2.0	34.0	83.0	101.0	127.0	90.0	67.0	60.0	51.0
4	Sand loam	0.2	5.0	14.0	53.0	58.0	71.0	57.0	55.0	63.0	55.0
5	Light sand loam	0.2	3.0	32.0	61.0	72.0	75.0	67.0	43.0	40.0	30.0
6	Clay loam	0.3	1.0	5.0	14.0	27.0	33.0	30.0	27.0	26.0	25.0
7	Peaty loam	2.0	3.0	6.0	28.0	34.0	58.0	48.0	31.0	29.0	24.0
8	Silt loam	0.4	2.0	21.0	55.0	67.0	78.0	52.0	28.0	25.0	25.0
9	Black loam	0.0	0.3	0.6	48.0	80.0	100.0	88.0	56.0	4.0	3.0
10	Very tight clay	1.0	2.0	4.0	44.0	55.0	69.0	63.0	36.0	40.0	32.0
11	Silt loam	2.0	3.0	14.0	53.0	55.0	71.0	37.0	36.0	33.0	11.0
12	Extra tight clay	1.0	2.0	2.0	2.0	8.0	13.0	12.0	12.0	11.0	9.0
13	Trenton fine loam	1.0	2.0	32.0	70.0	94.0	126.0	124.0	104.0	75.0	60.0
14	Fine silt loam	4.0	6.0	20.0	60.0	62.0	86.0	44.0	45.0	45.0	41.0
15	Light sandy loam	0.9	14.0	42.0	61.0	74.0	109.0	103.0	39.0	39.0	36.0
16	Medium sand loam	0.0	0.4	9.0	31.0	41.0	50.0	48.0	44.0	39.0	41.0
17	Sand	0.7	19.0	23.0	40.0	57.0	74.0	71.0	51.0	41.0	30.0
18	Light mountain loam	3.0	16.0	52.0	109.0	126.0	127.0	100.0	61.0	55.0	55.0
19	Loose, light mountain loam	3.0	6.0	88.0	115.0	123.0	126.0	79.0	50.0	47.0	44.0
20	Fine sand	0.5	21.0	35.0	47.0	64.0	72.0	42.0	39.0	31.0	32.0
21	Organic loam	2.0	3.0	48.0	93.0	107.0	116.0	96.0	72.0	55.0	41.0
22	White clay loam	3.0	5.0	38.0	51.0	70.0	81.0	46.0	46.0	47.0	47.0
Average		1.3	7.4	28.0	57.9	70.4	86.6	67.6	48.6	42.0	38.1

The soils show a wide variation in ammonifying powers. Although the quantity of sand present has a marked effect upon ammonification yet the predominating factor in these soils is the quantity of organic matter present. Those soils which had received a heavy dressing of manure are the ones which showed the most active bacterial changes. No. 12, the extra tight clay, never produced over 13 mgm. of ammonia, whereas no. 1, a clay loam, heavily manured, produced 135 mgm.

The quantity of ammonia produced when the water added is 10 per cent of the soil's water-holding capacity is very low in each case. When the water is raised to 20 per cent, ammonification becomes quite active in the lighter soils, but in the heavier soils there is a very slight increase. There is a gradual increase in ammonification as the water added increased up to 60 per cent. At this point every soil gives its maximum ammonification. The great variation in the physical composition of the soils and the large number of determinations which have been made would render it quite certain that maximum ammonification occurs in soils which contain water equal to 60 per cent of their water-holding capacity. This stated as the per cent of water in the soil would show a marked variation in the different soils, being very low in the case of the sand and high in the case of the organic loam. The lowest would be in the case of soil 17 which produced its maximum quantity of ammonia when it contained 18.6 per cent of water, whereas no. 22 produced its maximum quantity of ammonia when it contained 46.8 per cent of water, or the quantity of water necessary to add to these two soils is over three times as much in one case as it is in the other for maximum ammonification. This would account for the marked differences reported by various investigators as to the water required by ammonifying bacteria in soil.

As the water added increases above 60 per cent there is a gradual decrease in ammonification. In no case is this abrupt and even in saturated soils there is rapid ammonification. Considering the maximum ammonia produced at 60 per cent of water, the quantity produced at the other moisture contents is shown in figure 1.

Nitrification. Long before the process of nitrification was known to be due to microörganisms the underlying principles governing the speed of the reaction had been investigated nationally by France, Germany and Sweden. Among other things, they had learned that there must be a certain proportion of water, and in order that the maximum yield of nitrates be obtained this must be diminished as the soil becomes richer in nitrates. As early as 1887 Deherain (6) found that the most active nitrification took place when the soil was allowed to become partially dry between the applications of water, and later (7) he found that there was a relationship between the speed of nitrification and the moisture content of fallow soil, the nitrification increasing with the water. Boussingault (47) taught that when soils contain as much as 60 per cent of water they lose in a few weeks the greater part of their nitrates. This teaching gave rise to the general belief that denitrification may take place to a great extent in soils, but recent work has amply demonstrated that it is only under extremely abnormal conditions that this becomes an important factor. For this reason literature bearing on this phase of the subject is not considered here.

Deherain and Demoussy (9) found that the bacterial action of a soil was at its maximum when a rich soil contained 17 per cent of water, but that it decreased if the proportion of water fell to 10 per cent or rose to 25 per cent.

With soils less rich in humus a somewhat higher proportion of water was necessary to retard oxidation to any marked degree.

The optimum moisture content for nitrification, according to Deherain (7), is 25 per cent. An insufficient supply of moisture checked both nitrification and nitrogen fixation. This occurred when the water had been reduced to

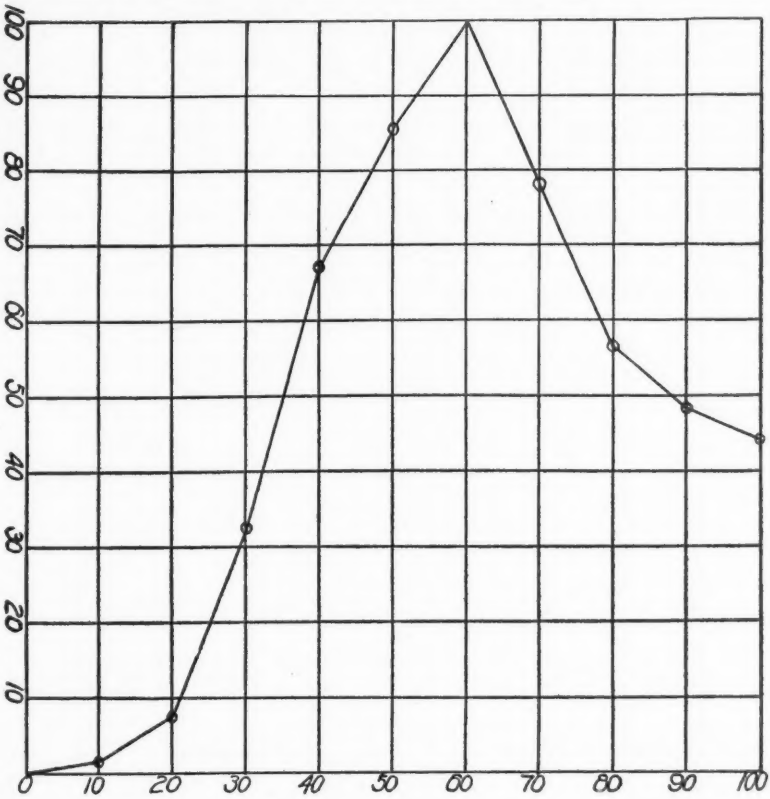

FIG. 1. AVERAGE PERCENTAGES OF AMMONIA PRODUCED IN SOILS RECEIVING VARYING QUANTITIES OF WATER.

The quantity produced at 60 per cent is taken as 100; on the ordinate is given the per cent of ammonia formed, whereas on the abscissa is given water applied as per cent of water-holding capacity.

16.5 per cent. This, however, would vary with the soil, for Schloesing (42) found bacterial activity less in fine-grained soils than in lighter, coarse-grained soil. In order that nitrification be equally active in both light and heavy soils the latter must have a higher percentage of water than the former, a difference in moisture content of the soil of 1 per cent, according to Dafert

and Bollinger (4), being sufficient to produce a marked change in the oxidation going on in the soil.

Fraps (13) found that the number of nitrifying organisms in a soil varies with the moisture and that their activity is periodic, rapid nitrification being preceded and followed by periods of less activity. Later he (14) found nitrification to be at its height in soil containing 55.6 per cent of its water-holding capacity. An excessive quantity of water practically stopped nitrification and was much more injurious than too small a quantity. The water requirements, however, varied considerably with the soil.

Coleman's work (3) with a loam soil showed nitrification to be most active when the soil contained 16 per cent of water. It was greatly retarded when the water content was reduced to 10 per cent or increased to 26 per cent. Not only nitrification but ammonification is dependent upon the moisture content of the soil. However, Lipman and Brown (32) found that ammonification in a loam soil increased with an increased water content even up to 35 per cent of the weight of the soil, but nitrification was most active in the same soil with a moisture content of 15 per cent, was only slightly less active with 10 per cent of moisture, and was still quite marked when the soil contained only 5 per cent of moisture. They found the greatest formation of nitrates to occur in soils when they were 50 per cent saturated with water. However, later Lipman, Brown and Owen (33) found ammonification to increase as the water added increased up to a certain percentage, which varied with the physical nature of the soil; but larger quantities of water reduced the ammonia recovered. Moreover, the work clearly demonstrates that the optimum moisture content for maximum ammonification is higher than it is for maximum nitrification.

Engberding (10) considered that the moisture content of a soil had a greater influence on numbers than did temperature, and the work of King and Doryland (27) clearly indicates that excessive soil moisture reduces both the number and the activity of soil bacteria.

Patterson and Scott's work (40) is interesting in that they found nitrification to be inactive in sand and clay soils which still contained about three times as much moisture as in their average air-dry condition. At the lower limits of moisture less water starts nitrification in sand than in clay. At the higher limits of moisture less water stops nitrification in sand than in clay, while the optimum amount of water probably varies for each soil. It is higher for clay, yet for both soils it lies within the range of from 14 to 18 per cent. A rise above the optimum amount of water is more harmful than an equal fall below it.

The work of the Utah Experiment Station (45) demonstrated that the application of irrigation water to a soil has a distinct beneficial effect upon nitrification, being greatest where 15 inches of water were applied when the nitric nitrogen formed amounted to 28.5 pounds per acre-foot of soil. The greatest benefit per inch of water, however, was obtained where only 7.5

inches of water were applied, resulting in 3.8 pounds of nitric nitrogen per inch of water, while where 15 inches of water were applied it was 1.1 pounds of nitric nitrogen per inch of water applied, and when 25 inches of water were applied to the soil the nitric nitrogen produced was only 0.7 pound.

Münter and Robson (38) found that horn meal decomposed more rapidly in dry, sandy soil than in clay or loam, while with a higher moisture content there was little difference. Ammonium-sulfate transformation increased with a higher water content. The best nitrate formation from horn meal occurred in sandy soils. In clay and loam it was best with a medium water content. Sharp (44) found that the water content most favorable for ammonification was not the optimum condition for nitrification. The former was most rapid with a 25 per cent water content and was not markedly affected by 3 per cent differences. Nitrification was at its maximum when the soil contained 19 per cent of water. When it was increased to 25 per cent, the rate of nitrification was decreased 50 per cent.

McBeth and Smith (36) found a slight variation in the bacterial number and nitrifying power of soils, depending upon the moisture content. However, Gainey (15) considers that among the factors controlling the bacterial activity of a soil the available moisture probably plays a leading part. But we (19) have reported results which indicate that the nitrous-nitrogen content of a soil is independent of the irrigation water applied up to 37.5 inches a year. Results recently published from the Utah Experiment Station (17) clearly demonstrate that the influence exerted by water upon ammonifying, nitrifying, and nitrogen-fixing activities of the soil varies greatly with the organic matter in the soil and is much more marked in effect on soils recently manured than on those which have received no manure.

However, in tests of soil receiving varying quantities of water in the field (20) no increase of nitric-nitrogen accumulation or increased nitrification was noted, as is seen from the following:

TREATMENT	NITRIC NITROGEN IN SOIL	NITROUS NITROGEN IN SOIL	NITRIFYING POWERS
	per cent	*per cent*	*per cent*
No water	100	100	100
15.0 inches	48	115	98
25.0 inches	51	62	98
37.5 inches	43	115	98

The decrease in nitric nitrogen may be due to a number of factors: (1) washing out by the water, (2) increased plant growth and hence the use of more nitrates, (3) increased bacterial activity which would transform nitrates into proteins. The figures in the third column would indicate that although the addition of water to a soil changes its nitrifying powers it is not permanent.

The same set of soils used in the previous section to determine the influence of water on the ammonifying power was used to study the nitrifying power.

The water varied from 10 to 100 per cent of the water-holding capacity of the soil. The results, as given in table 5, are the average of six of more closely agreeing determinations.

The soils show a wide variation in their nitrifying power, yet all are quite active, as would be expected of them since they are well supplied with potassium, phosphorus, and other elements essential to bacterial growth, with the exception of nitrogen. This element in the case of the unmanured soils is

TABLE 5]

Nitric nitrogen formed in 100 gm. of soil maintained at various moisture contents expressed in per cent of water-holding capacity

SAMPLE NUMBER	KIND OF SOIL	NITRIC NITROGEN FORMED							
		10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent	80 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	Clay loam	10.2	16.5	28.0	60.5	65.2	85.3	7.5	4.0
2	Clay loam	0.4	7.8	21.5	32.0	38.0	17.5	0.8	1.2
3	Tight clay loam	1.0	3.9	10.8	58.0	92.3	76.4	2.4	2.2
4	Sand loam	3.6	6.1	20.7	26.0	81.1	94.1	7.4	4.6
5	Light sand loam	3.7	7.0	11.7	21.9	51.6	20.0	15.7	3.1
6	Clay loam	2.9	4.3	10.0	34.7	77.5	105.2	38.1	2.9
7	Peaty loam	0.0	0.4	5.5	31.9	72.2	142.7	36.9	3.7
8	Silt loam	6.5	11.2	25.0	67.8	89.7	73.8	11.6	2.4
9	Black loam	3.5	5.0	12.0	27.2	45.8	52.4	49.9	7.1
10	Very tight clay	6.0	8.4	14.1	60.1	59.4	99.7	40.2	4.0
11	Silt loam	3.5	9.2	34.0	59.9	60.4	61.5	27.0	2.2
12	Extra tight clay	10.9	11.6	11.8	11.7	12.3	3.0	3.0	3.7
13	Trenton fine loam	12.1	18.4	39.2	60.3	79.4	65.9	29.8	13.8
14	Fine silt loam	23.9	24.5	27.7	28.9	39.2	121.4	57.4	18.1
15	Light sandy loam	17.0	25.2	25.5	25.7	26.4	20.3	19.2	18.3
16	Medium sand loam	0.7	2.2	10.6	16.8	29.2	101.6	90.4	2.5
17	Sand	2.8	3.8	4.0	5.9	6.8	3.6	2.4	2.1
18	Light mountain loam	6.3	13.3	27.0	51.4	53.8	26.1	2.5	2.3
19	Loose, light mountain loam	4.4	8.9	10.6	11.6	24.0	25.0	6.0	4.3
20	Fine sand	6.0	5.6	5.6	5.4	4.3	3.5	3.6	3.6
21	Organic loam	3.8	6.9	18.7	40.7	56.0	56.7	40.7	9.4
22	White clay loam	8.5	13.2	12.7	42.7	20.6	6.1	5.9	6.0
Average		6.3	9.7	17.6	35.5	49.3	57.4	22.7	5.5

low. All contain large quantities of calcium and magnesium carbonate. As an average, those soils which showed a low ammonifying power also show a comparatively low nitrifying power. It is interesting to note that nitrification is comparatively more active with the 10 per cent of water than is ammonification. However, the addition of water to the soil greatly accelerates nitrification. Although the quantity of nitric nitrogen accumulating in the soil progressively increases with increasing water up to a certain point, yet it

is much more pronounced in some soils than in others. About half of the soils produce maximum quantities of nitric nitrogen at 50 per cent saturation, whereas the others produce most at 60 per cent. This probably indicates that optimum moisture for maximum nitrification in soils lies somewhere between 50 and 60 per cent of saturation, thus indicating, as has been

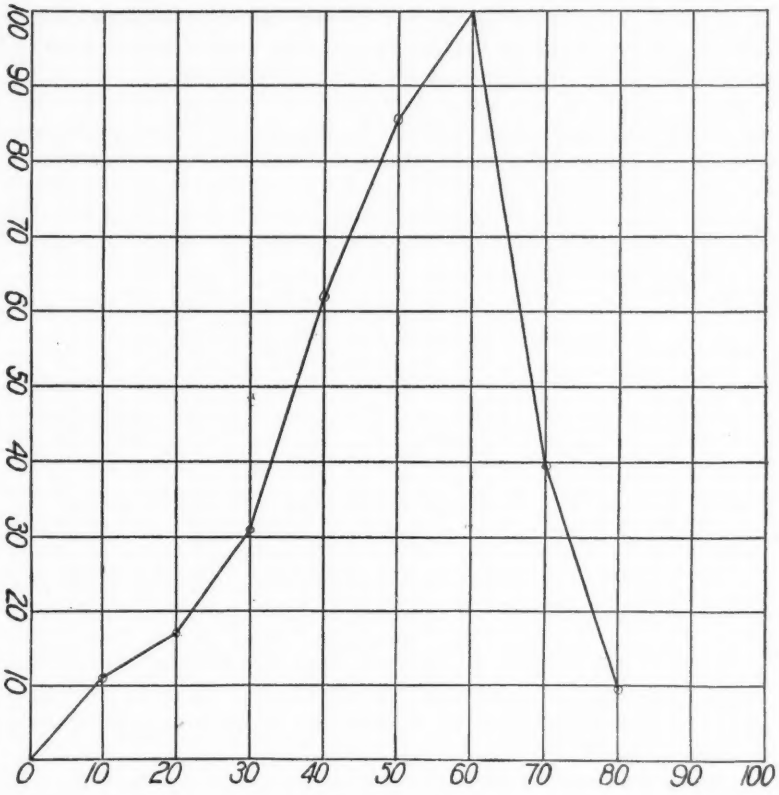

FIG. 2. AVERAGE PERCENTAGES OF NITRIC NITROGEN PRODUCED IN SOIL RECEIVING VARIOUS QUANTITIES OF WATER

The quantity produced at 60 per cent is taken as 100; on the ordinate is given the per cent of nitric nitrogen formed, whereas on the abscissa is given water applied as per cent of water-holding capacity.

the finding of many other workers, that the optimum moisture content of soil for best nitrification is somewhat lower than is required for optimum ammonification. When this is considered from the viewpoint of water-holding capacity the results are remarkably uniform as compared with the results

reported by most previous workers. These results, therefore, make it clear that the more logical method is to consider moisture from the standpoint of water-holding capacity of the soil and not from its percentage in the soil. The average results are given graphically in figure 2.

When the water content of the soil exceeds 60 per cent there is a rapid decrease in the nitric-nitrogen content. All soils ceased to nitrify when saturated; it was very slight at the 90 per cent of the water-holding capacity and in most soils less nitric nitrogen was produced where the water content was 80 per cent of saturation than where it was 10 per cent.

It is interesting to note the uniform correlation between ammonification and nitrification. Examining the results in tables 4 and 5 we find that wherever ammonification is higher at 70 per cent saturation than it is at 50 per cent the optimum for nitrification in that soil is at 60 per cent of the water-holding capacity and conversely, wherever ammonification is higher at 50 per cent than it is at 70 per cent, the optimum moisture for maximum nitrification is at 50 per cent.

INFLUENCE OF WATER ON NITROGEN FIXATION

Azotobacter are very resistant to drying; they may be dried for a considerable time in a desiccator over sulfuric acid. Pure cultures are just as resistant to drying as are mixed cultures (25). This would vary somewhat with the media in which the bacteria are dried, for the survival of non-spore-forming bacteria in air-dry soil is due in part to the retention by the soil of moisture in the hygroscopic form. This however, is not the only factor, for the longevity of bacteria in a solid is not directly proportional to its grain size and hygroscopic moisture. Giltner and Langworth (16) found that bacteria resisted desiccation longer in a rich clay loam than in sand. Furthermore, if bacteria are suspended in the extract from a rich clay loam before being subjected to desiccation in sand they live longer than if subjected to dessication after suspension in a physiological salt solution. Because of this they consider that soils contain substances which have a protective influence upon bacteria subjected to desiccation.

Lipman and Burgess (30) have found that many soils manifest a vigorous nitrogen-fixing power even after being air-dried and kept in stoppered museum bottles for periods varying from 5 to 20 years. In some cases the fixation was equally as high as in freshly-collected samples. The organisms from such soils are more easily attentuated than are other organisms which have not been so dried (50). The tendency is for soils gradually to decline in nitrogen-fixing power on drying. This may manifest itself as early as the second week.

During the periods of drying the organisms are inactive as they require moisture for growth and reproduction. For maximum nitrogen-fixation a definite moisture content is needed. Warmbold (48) found the optimum

moisture content to be 20 per cent. When it was below 10 per cent there was no nitrogen fixed, and in some cases there was a decided loss of nitrogen. Krainski (28) allowed soil with varying moisture content to stand for some time and then inoculated it into mannite solutions and obtained maximum fixation in the soils containing fairly small quantities of water. Later, however, he decided that soil should be damp—but not wet—and well aerated for maximum nitrogen fixation. The water requirements vary with different soils. As a general rule the higher the humus content of the soil the more water will be required for optimum nitrogen fixation (29). The quantity of water present may, however, become so great that it may kill all *Azotobacter* in addition to stopping nitrogen fixation (11).

An insufficient supply of moisture checks both nitrification and nitrogen fixation (8). This occurs in some soils when the water content has been reduced to 16.5 per cent. This again varies with the soil, for Schloesing (42) found bacterial activity less in fine-grained soils than in lighter, coarse-grained soils. A difference in moisture content of 1 per cent, according to Defert and Bollinger (4), is sufficient to produce a marked change in the oxidation going on in the soil.

The moisture requirement of the nitrogen-fixing bacteria, according to Lipman and Sharp (31) is more nearly that of the ammonifying than of nitrifying organisms. In a sandy loam it was found to vary between 20 and 24 per cent of moisture in the soil. At the higher percentages of moisture up to 24 per cent the anaerobic nitrogen fixers are most active, but the action of the aerobes is slightly depressed. Thus, in many soils two maxima of nitrogen fixation occur, depending upon whether the conditions are favorable for the anaerobic or aerobic organisms.

Traaen's results (46) differ from Lipman's in showing only the one maximum, as is seen from the following which gives the milligrams of nitrogen fixed in 100 gm. of soil.

TEMPERATURE	PER CENT OF WATER				
	5	10	17.5	25	30
°C.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
13	0.1	1.5	11.2	13.4	5.4
25	1.9	1.9	13.2	16.6	15.5

He used a loam soil with a maximum water capacity of 27.4 per cent. It is quite evident from his statement that anaerobic organisms played a prominent part in the fixation at the higher moisture contents.

Since the carbohydrates disappeared much more rapidly in the soils containing the greater quantities of water, it is quite possible that greater quantities of nitrogen per gram of carbohydrate consumed are fixed where the smaller quantities of water are applied. This, together with the different

methods used by the several investigators, would explain the apparent discrepancy in their results.

In a series of pot experiments in which a calcareous loam receiving various amounts of water was used the author (17) found the moisture content for maximum nitrogen fixation to lie between 15 and 22 per cent. These results also brought out the two maxima which were first noted by Lipman. These soils were kept at the various moisture contents for four months. All were then incubated at 28°C. for 21 days with a moisture content of 20 per cent.

TREATMENT	NITROGEN FIXED
per cent of water added	*per cent*
12.5	100
15.0	108
17.5	102
20.0	104
22.5	108

In this soil the optimum for the aerobes would appear to be at 17.5 per cent and that for the anaerobes 22.5 per cent or higher.

When too large a quantity of water is applied there is a tendency to depress the total nitrogen fixed, as is illustrated by the following results in which various quantities of water were applied to a soil throughout the year under field conditions (20).

37.5 inches of water applied during summer; 1.4 mgm. of nitrogen fixed in 100 gm. of soil
25.0 inches of water applied during summer; 2.1 mgm. of nitrogen fixed in 100 gm. of soil
15.0 inches of water applied during summer; 8.5 mgm. of nitrogen fixed in 100 gm. of soil
No water applied during summer; 3.5 mgm. of nitrogen fixed in 100 gm. of soil

The maximum for anaerobic conditions does not appear in these results probably because the soil did not become filled with water, since under field conditions the water rapidly drains away or is evaporated.

The same 22 soils as were used in the ammonifying and nitrification tests were used to study the influence of water on nitrogen fixation. The moisture was kept at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per cent of the water-holding capacity and the results as given in table 6 are the average of six or more closely agreeing determinations.

There is a wide variation in the nitrogen-fixing powers of the soil. Some are very active, as for instance no. 7, the peaty loam, and no. 21, the organic loam. In reality it would appear that the soils of this series with the greatest quantity of organic matter are most active as nitrogen fixers. One soil, no. 22, the white clay loam, lost nitrogen with each concentration of water. This was uniformly true in the sixty determinations made on this soil. Five

other soils, no. 4, 6, 8, 18 and 19, lost nitrogen with some concentrations of water and gained with the other concentrations. It is interesting to note that the loss is usually at the lower water contents, whereas at higher water contents there is a gain of nitrogen. This gain usually appears when enough water has been added to produce anaerobic conditions in the soil. From this one must conclude that the anaerobes are the principal azofiers in these soils. Only one soil (no. 16) fixed nitrogen with a low water content and lost

TABLE 6

Nitrogen fixed in 100 gm. of soil maintained at various moisture contents expressed in per cent of water-holding capacity

SAMPLE NUMBER	KIND OF SOIL	NITROGEN FIXED									
		10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent	80 per cent	90 per cent	100 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	Clay loam.....	2.4	4.5	7.8	8.7	2.8	7.5	4.2	5.9	0.0	−1.4
2	Clay loam.....	6.7	4.7	3.9	3.6	11.5	10.3	5.9	3.5	5.0	4.2
3	Tight clay loam..	4.2	10.9	13.7	19.6	9.2	10.0	12.6	8.9	7.0	9.4
4	Sand loam.....	−8.9	−6.4	−6.1	−1.9	−2.1	8.1	10.0	12.0	3.0	0.0
5	Light sand loam.	1.9	5.2	6.7	3.8	2.2	4.9	3.9	12.9	3.6	−1.1
6	Clay loam.....	3.1	−3.0	−2.2	0.2	−0.3	1.2	8.4	3.9	−1.4	−2.1
7	Peaty loam.....	9.8	−3.5	−9.8	−3.9	0.0	11.0	17.7	19.6	10.9	2.6
8	Silt loam.....	−12.2	−19.1	−15.1	−14.8	−5.7	−7.3	−1.4	2.2	−4.0	−16.9
9	Black loam.....	0.1	2.1	−0.8	−6.8	−0.2	0.6	3.0	3.0	1.4	2.1
10	Very tight clay..	3.7	2.2	3.4	0.5	2.5	6.9	3.5	5.9	5.6	6.8
11	Silt loam.....	4.6	4.7	−0.1	0.5	3.5	8.1	4.2	8.7	6.0	2.6
12	Extra tight clay..	3.9	0.8	2.0	−0.5	1.3	5.2	2.4	4.8	4.2	5.6
13	Trenton fine loam	2.1	3.0	5.5	5.6	8.2	16.8	16.8	4.6	6.8	5.6
14	Fine silt loam...	9.1	10.8	9.1	1.4	7.2	5.3	7.7	1.4	3.5	4.4
15	Light sandy loam	7.3	9.2	4.4	5.8	11.0	9.5	13.7	5.6	5.8	6.6
16	Medium sand loam.....	3.5	3.8	4.2	5.6	1.4	0.2	5.6	−1.0	−7.5	−6.3
17	Sand.....	7.0	4.2	0.8	0.3	1.4	1.1	0.3	2.1	1.4	2.3
18	Light mountain loam.....	−7.8	−2.0	1.1	3.6	3.4	5.3	9.5	14.2	1.5	2.4
19	Loose light mountain loam.....	−2.3	−1.4	−1.7	−2.1	0.8	7.6	8.0	3.9	1.1	0.3
20	Fine sand.....	4.3	3.4	3.9	3.8	8.9	11.5	9.6	9.8	7.1	6.2
21	Organic loam....	10.1	10.0	9.5	12.6	6.2	−0.7	16.4	16.1	10.9	7.0
22	White clay loam.	−3.5	−3.7	−0.4	−1.4	0.4	−1.0	−2.2	−2.8	−0.3	−0.7

with a high content. It is interesting to note that the mere changing of the water content of a soil changes it from one which is gaining nitrogen to one which is losing. This loss usually occurs under aerobic and not anaerobic conditions as is usually considered requisite for denitrification. The question naturally arises—Can it be associated with the rapid burning out of the organic matter of the soil which is so characteristic of some arid soils? This being the case the question arises as to the specific organisms which bring about the change.

Most soils show a maximum fixation for a specific water content. This, however, varies widely with the soil. In many cases the two maxima appear, —the one when the water content is from 40 to 60 per cent, the other when it is 70 to 100 per cent. This is undoubtedly due to the two groups of organisms—in the first case to the aerobes and in the second to the anaerobes. The average for all soils shows a maximum nitrogen fixation when the soils contain 70 per cent of their water-holding capacity. The average results, considering that containing 70 per cent of water as 100 per cent, are shown graphically in figure 3.

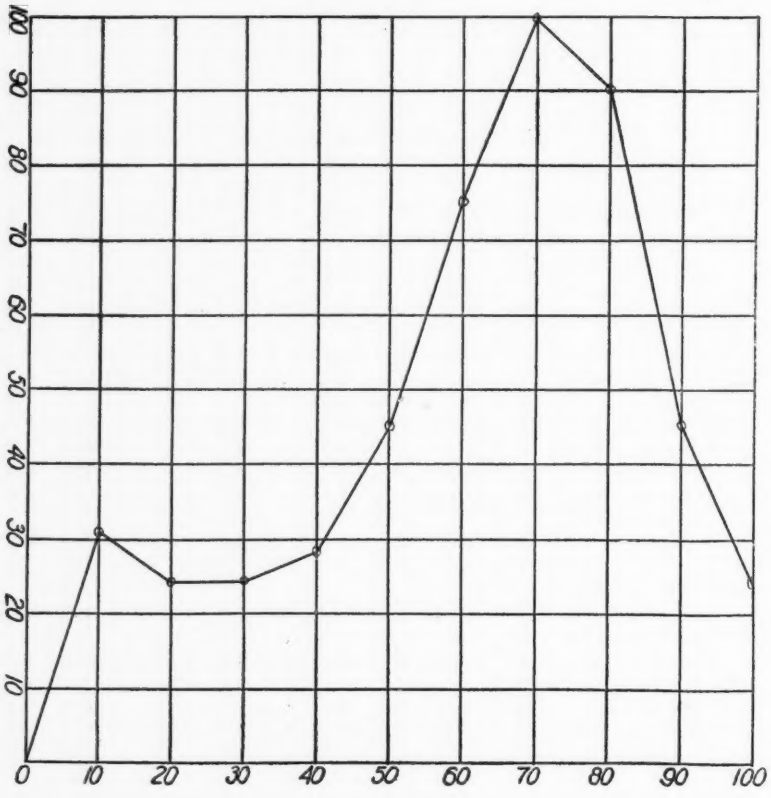

FIG. 3. AVERAGE PERCENTAGE OF NITROGEN FIXED BY SOILS RECEIVING VARYING QUANTITIES OF WATER

The quantity fixed at 70 per cent is taken as 100; on the ordinate is given the per cent of nitrogen fixed, whereas on the abscissa is given water applied as per cent of water-holding capacity

In the main, most soils show a low fixation at the first three water contents and this averages practically the same with 10, 20, 30 and 40 per cent of the water-holding capacity. A few soils fix fair quantities of nitrogen with only 10 per cent of water. The fixation at 90 and 100 per cent is uniformly low, but the very few that show a loss of nitrogen at these water contents would make it appear that water does not favor denitrification in these soils.

RELATIONSHIP BETWEEN AMMONIFICATION, NITRIFICATION, AND NITROGEN FIXATION

The comparative results for ammonification, nitrification, and nitrogen fixation are shown graphically in figure 4.

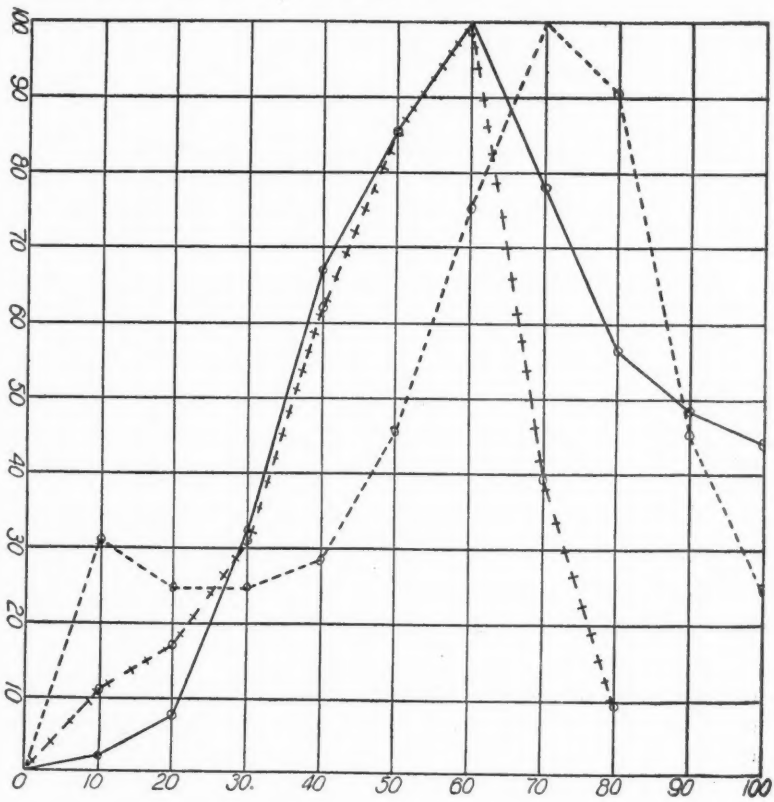

FIG. 4. AVERAGE PERCENTAGES OF AMMONIA ———— AND NITRIC NITROGEN ++++ FORMED AND NITROGEN FIXED – – – – IN SOIL RECEIVING VARYING QUANTITIES OF WATER

On the ordinate is given the per cent increase of the respective substances and on the abscissa the quantity of water applied as per cent of water-holding capacity

The point at which maximum activity was obtained has been taken as 100 per cent. Both ammonification and nitrification are at their maximum, according to these results, when the soil contains 60 per cent of the water-holding capacity. However, the individual results showed that it is between 50 and 60 for nitrification and 60 for ammonification.

Only one maximum appears in the average results for nitrogen fixation. This is at 70 per cent and probably favors anaerobic in place of aerobic bacterial activity.

The nitrogen-fixing bacteria do not respond in activity to different water treatments as readily as do the other bacteria. This is probably due to the heterogenous group of organisms which bring about this change in counter-distinction to the homogenous group bringing about nitrification. Eighty per cent of the water-holding capacity reduces nitrogen fixation to 90.4 per cent, ammonification to 56.1 per cent, and nitrification to 9.6 per cent.

BACTERIAL ACTIVITY VS. CROP PRODUCTION

The optimum moisture content for nitrification is not far from the optimum for field crops in general. Wollny (52) placed this at from 60 to 80 per cent of the water-holding capacity of the soil. Mayer (35) placed the optimum moisture content of wheat at 80 per cent of the water-holding capacity of the soil, rye at 75 per cent, barley at 75 per cent, and oats at 85 to 90 per cent.

TABLE 7

Water-holding capacity of soils at which maximum crops were obtained

INVESTIGATOR	CROP	PER CENT OF WATER-HOLDING CAPACITY AT WHICH MAXIMUM YIELD WAS OBTAINED
Harris and Maughan (23)	Wheat	60
Harris (22)	Alfalfa	60
Fittbogen (12)	Oats	60–80
Hellriegel (24)	Barley	60
Schroeder	Barley	40–60
Daszenski (5)	Potatoes	Better at 58 than at 33
von Sellhorst (43)	Oats	64–74
Kiesselbach (26)	Corn	60
Maercker (34)	White mustard	60
Wilms (51)	Potatoes	Better at 80 than at 58 or 33
Ohlmer (39)	Wheat	Better at 70 than at 45

However, the majority of investigators place the optimum moisture for maximum production with most plants at from 60 to 70 per cent, as may be seen from the summarized results in table 7.

The different methods used by the several investigators would account in a measure for the variation in the results reported. Those by Harris, how-

ever, were obtained by the same method as was used in the bacteriological investigations. These, taken in connection with those of the other investigators, indicate that there is a close correlation between the metabolism of the beneficial bacteria and of the higher plants in so far as their water requirements are concerned.

OTHER SOIL CONSTANTS AND THEIR RELATIONSHIP TO BACTERIAL ACTIVITIES

The moisture equivalents of the various soils were determined by the method of Briggs and McLane (1). These, together with the per cent of water necessary for maximum ammonification, are given in table 8.

TABLE 8

Moisture equivalent of soils and per cent of moisture for maximum ammonification

SAMPLE NUMBER	DESCRIPTION	MOISTURE EQUIVALENT	HYDROSCOPIC COEFFICIENT	PER CENT MOISTURE FOR AMMONIFICATION	a_1	a_4
17	Sand	3.32	0.39	18.6	1.807	15.40
20	Fine sand	5.08	1.15	19.8	1.417	6.26
15	Light sandy loam	11.38	3.61	26.4	1.213	3.82
18	Light mountain loam	20.46	4.70	30.6	0.880	3.83
13	Trenton fine loam	21.75	3.61	30.6	0.828	4.99
5	Light sand loam	23.12		31.8	0.830	
11	Silt loam	25.54	4.82	33.6	0.822	4.36
19	Loose light mountain loam	18.32	4.91	33.6	1.146	4.28
6	Clay loam	29.18		34.2	0.740	
9	Black loam	31.47	6.06	35.4	0.725	3.76
16	Medium sand loam	29.36	5.92	36.0	0.797	3.95
10	Very tight clay	39.02	5.57	36.6	0.615	4.32
12	Extra tight clay	45.15	7.77	36.6	0.532	3.09
2	Clay loam	20.65	3.78	36.6	1.162	6.35
4	Sand loam	28.14	4.39	37.2	0.874	5.64
3	Tight clay loam	28.33	4.42	39.0	0.932	5.97
1	Clay loam	23.35	4.24	40.8	1.208	6.65
7	Peaty loam	32.41	6.55	44.4	0.981	4.86
8	Silt loam	30.78	6.66	46.2	1.092	5.04
14	Fine silt loam	35.45	7.83	46.8	0.965	4.37
21	Organic loam	34.02	9.94	46.8	1.005	3.44
22	White clay loam	27.20	8.00	46.8	1.257	4.28

According to Briggs the moisture-holding capacity, the wilting coefficient, the moisture equivalent and the hygroscopic coefficient are related by linear equations thus:

$$c = 2.9\ w + 21.$$
$$c = 1.57\ e + 21.$$
$$c = 4.26\ h + 21.$$

Where c is written for the moisture capacity as defined by Hilgard, w for wilting coefficient, e for moisture equivalent, and h for hygroscopic coefficient.

If therefore the optimum moisture for maximum bacterial activity is directionally proportional to c (in the case of the ammonifiers $c = 0.6$) then these other soil constants may also be related to these constants by a similar set of linear equations.

$$M_a = a_1 e + 12.6, \text{ whence } a_1 = \frac{M_a - 12.6}{e}$$

$$M_a = a_2 w + 12.6, \text{ whence } a_2 = \frac{M_a - 12.6}{w}$$

$$M_a = a_3 h + 12.6, \text{ whence } a_3 = \frac{M_a - 12.6}{h}$$

Writing M_a for the per cent of water for maximum ammonification and a_1, a_2, and a_3 constants, calculated these become $a_1 = 0.942$, $a_2 = 1.74$ and $a_3 = 2.555$; that is the moisture requirement for maximum ammonification may be obtained from any of the soil constants by the following equations:

$$M_a = 0.6\, c$$
$$M_a = 0.942\, e + 12.6$$
$$M_a = 1.74\, w + 12.6$$
$$M_a = 2.55\, h + 12.6$$

a_1 as given in table 8 and calculated from the moisture equivalent, varies from 0.615 to 1.807 with a mean of 0.992. The irregular variation of a_1, as determined from the moisture equivalent, makes it appear evident that the relationship between the moisture equivalent and moisture requirements for maximum ammonification is not as well defined as is the relationship between water-holding capacity and water requirements for maximum ammonification.

The value of a_3 as calculated from the determined hygroscopic moisture varies from 3.09 to 15.4 with a mean of 5.24, thus giving results invariably higher than those obtained by calculating from the Briggs formula.

The relationship between the moisture equivalent and the per cent of moisture for maximum nitrification is shown in table 9.

A set of equations similar to those written for ammonification may be written for nitrification thus, in which the optimum moisture for maximum nitrification is taken at 0.55:

$$M_n = a_1 e + 11.55, \text{ whence } a_1 = \frac{M_n - 11.55}{e}$$

$$M_n = a_2 w + 11.55, \text{ whence } a_2 = \frac{M_n - 11.55}{w}$$

$$M_n = a_3 h + 11.55, \text{ whence } a_3 = \frac{M_n - 11.55}{h}$$

Calculated, these constants become $a_1 = 0.8525$, $a_2 = 1.472$ and $a_3 = 2.163$. Therefore, the moisture requirements for maximum nitrification may be obtained from any of the soil constants by the following equations:

$$M_n = 0.55\, c$$
$$M_n = 0.8525\, e + 11.55$$
$$M_n = 1.472\, w + 11.55$$
$$M_n = 2.163\, h + 11.55$$

a_1 as determined from the moisture equivalent varies from 0.396 to 1.208 with a mean of 0.806. This shows a much wider variation from the moisture equivalent than from the maximum water-holding capacity.

TABLE 9

Moisture equivalent of soils and per cent of moisture for maximum nitrification

SAMPLE NUMBER	DESCRIPTION	MOISTURE EQUIVALENT	PER CENT OF MOISTURE FOR MAXIMUM NITRIFICATION	a_1
17	Sand	3.32	15.5	0.873
20	Fine sand	5.08	19.8	1.023
19	Loose light mountain loam	18.32	33.6	1.147
15	Light sandy loam	11.38	22.0	0.826
18	Light mountain loam	20.46	25.5	0.630
13	Trenton fine loam	21.75	25.5	0.593
5	Light sand loam	23.12	26.5	0.601
11	Silt loam	25.54	33.6	0.822
6	Clay loam	29.18	34.2	0.749
9	Black loam	31.47	35.4	0.725
16	Medium sand loam	29.36	36.0	0.797
10	Very tight clay	39.02	36.6	0.615
12	Extra tight clay	45.15	30.5	0.396
2	Clay loam	20.65	30.5	0.867
4	Sand loam	28.14	37.2	0.876
3	Tight clay loam	28.33	32.5	0.702
1	Clay loam	23.35	40.8	1.208
7	Peaty loam	32.41	44.4	0.981
8	Silt loam	30.78	38.5	0.965
14	Fine silt loam	35.45	46.8	0.965
21	Organic loam	34.02	46.8	1.005
22	White clay loam	37.20	31.2	0.500

Similar equations may be written for nitrogen fixation in which we find the value of $a_1 = 1.049$, $a_2 = 1.947$ and $a_3 = 2.848$. That is, the moisture requirements for maximum azofication may be obtained from any of the soil constants by the following equations:

$$M_{az} = 0.70\ c$$
$$M_{az} = 1.049\ e + 14.7$$
$$M_{az} = 1.947\ w + 14.7$$
$$M_{az} = 2.848\ h + 14.7$$

SUMMARY

The influence of water upon the bacterial activities of 22 soils were studied. They represent the typical farming lands of Cache Valley—dry land, irrigated, manured and unmanured. They range all the way from a loose sand to a very tight clay and from soils nearly devoid of organic matter to others very rich in organic material. Their moisture-holding capacity varied from 31 to 78 per cent and was closely correlated with the quantity of clay and organic material. Their moisture equivalent varied from 3.32 to 45.15 and the wilting coefficient, as calculated from the moisture equivalent, from 1.80 to 24.54.

Every soil gave a maximum ammonification when it contained 60 per cent of its water-holding capacity of water. Nitrification was at its maximum at 50 or 60 per cent and varied with specific soils. Many of the soils showed two maxima for nitrogen fixation—one at from 50 to 60 and the other from 70 to 80.

The average comparative results for ammonification, nitrification, and nitrogen fixation were as follows:

	PER CENT OF MOISTURE									
	10	20	30	40	50	60	70	80	90	100
Ammonification	1.5	8.5	32.4	66.9	81.3	100.0	78.1	56.1	48.5	44.0
Nitrification	11.0	16.9	30.7	61.9	85.9	100.0	36.6	9.6		
Nitrogen fixation	31.5	24.7	24.7	27.4	45.2	75.3	100.0	90.4	45.2	24.7

Using the formula of Briggs for the moisture equivalent, and the wilting and hygroscopic coefficients, we may write the following equations as representing approximately the water requirements for maximum bacterial activity, where c is written for the moisture capacity as defined by Hilgard, w for the wilting coefficient, e for the moisture equivalent, and h for the hygroscopic coefficient:

$$M_a = 0.6\ c$$
$$M_a = 0.942\ e + 12.6$$
$$M_a = 1.74\ w + 12.6$$
$$M_a = 2.55\ h + 12.6$$
$$M_n = 0.55\ c$$
$$M_n = 0.8525\ e + 11.55$$

$$M_n = 1.472\,w + 11.55$$
$$M_n = 2.163\,h + 11.55$$
$$M_{az} = 0.7\,c$$
$$M_{az} = 1.049\,e + 14.7$$
$$M_{az} = 1.947\,w + 14.7$$
$$M_{az} = 2.848\,h + 14.7$$

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NUTRIENT REQUIREMENT OF THE POTATO PLANT GROWN IN SAND CULTURES TREATED WITH "TYPE I" SOLUTIONS

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INTRODUCTION

Recent experimental work dealing with the fundamental problem of plant nutrition has been directed mainly to such crop plants as wheat, barley, rice, buckwheat and soybeans grown in water and sand cultures. The potato, an entirely different type of plant and one that presents a number of special problems, has received but little attention. Skinner (13) suggested a method for growing potato plants in water cultures, but apparently little has since been done along this line. Johnston (5) called attention to some of the difficulties encountered in such a study and reported some preliminary work on the nutrient requirement of the potato plant. It was found that fairly uniform sprouts could be obtained for water and sand culture studies from tubers planted in a bed of sawdust. These sprouts did not grow well in water cultures although such a medium is ideal chemically. The medium used in the experiments reported in the present paper was pure white quartz sand treated with the nutrient solutions under consideration.

Two series of experiments were undertaken to determine the best proportion of salts necessary to produce the best growth of potato plants and the greatest yield of tubers. Irish Cobbler was the variety of potato used throughout these experiments.

SERIES I

Introductory

The abnormal growth of the potato plant in water cultures made it impractical to carry on any extensive salt requirement studies where such a medium was used. Sand cultures were therefore selected for these experiments. There are, however, several objections to sand culture methods as often practiced. A change in the moisture content of the sand takes place during the interval between solution renewals. A constant renewal of solutions would overcome this objection, but such a practice is not feasible. There is also a difference in the moisture content of the sand between the beginning and the end of the experiment, even when the cultures are daily brought back to their original weight by the addition of water. As the plant grows, a part of the

original total weight (mostly water) of the culture is transferred from the sand to the plant. Each time the culture is brought back to its original weight, the total weight is of course the same as the original total weight, but the sand mass is lighter and the plant heavier. With each successive operation the moisture content of the sand becomes less where the plant gains in weight. The weight of the moisture lost from the sand and not the amount lost from the entire culture (plant and sand mass) is the quantity to be added at each renewal. This error is worth noting where plants are used whose weight increases greatly in proportion to the weight of the solution in the sand mass. Other changes are brought about by the selective absorption of elements and ions. With these limitations in mind the sand culture method was employed as best suited to the conditions of the present study.

Procedure and method of experimentation

Potato tubers (4-oz. size) with sprouts just beginning to develop were selected from a lot of home-grown Irish Cobblers and planted 3 to 4 inches deep in sawdust in one of the greenhouses of the Maryland Agricultural Experiment Station on October 8, 1919. About 7 weeks later (November 25) the sprouts were separated from their tubers, washed in tap water and divided into three groups according to size and development. Group *A* was composed of sprouts with 6 to 8 leaves well developed, but not of full size, group *B* was composed of sprouts with 4 or 5 leaves well started and group *C* of sprouts with 1 or 2 leaves started or with leaves just beginning to open from their buds. These sprouts were then washed in distilled water.

Three sprouts with well developed roots, one from each group, were weighed and placed in a 1-gallon glazed earthenware jar containing 4500 gm. of air-dry sand[1] and 1000 cc. of nutrient solution. The sand was then flooded by adding 500 cc. more of the nutrient solution, thus making the final adjustment of the plants in the sand very easy. Enough of the solution was then drawn off to bring the level of the water-table below the surface of the sand. The following morning more of the solution was withdrawn to reduce the total amount to 675 gm. There were then 675 gm. of solution to 4500 gm. of air-dry sand, or 15 per cent of the dry weight of the sand was the weight of the solution in the culture. A small collar of cotton was placed around the stem of each plant at the surface of the sand and a wax seal (4 parts parawax and 1 part white vaseline by weight) similar to that used by Briggs and Shantz (1) was poured over the surface of the sand at a temperature of 50° to 60°C. The cotton served the double purpose of protecting the plants from the hot wax and providing space for the transverse growth of the stems. Twenty-two cultures were thus prepared, weighed and placed on a rotating table similar to that employed by Shive (11). An atmometer corrected to the Livingston

[1] The sand used in the experiments of series I was not washed since it was relatively free from impurities.

(6) standard spherical atmometer was operated on the table with the cultures and a maximum-minimum thermometer was suspended in the shade beneath the table.

The method of renewing solutions differed somewhat from those employed by McCall (7), McCall and Richards (8) and Shive and Martin (12). Each pot was provided with a glass tube, of about 4-mm. bore, extending to the bottom where it made a 90° angle and ended in a funnel-shaped opening.

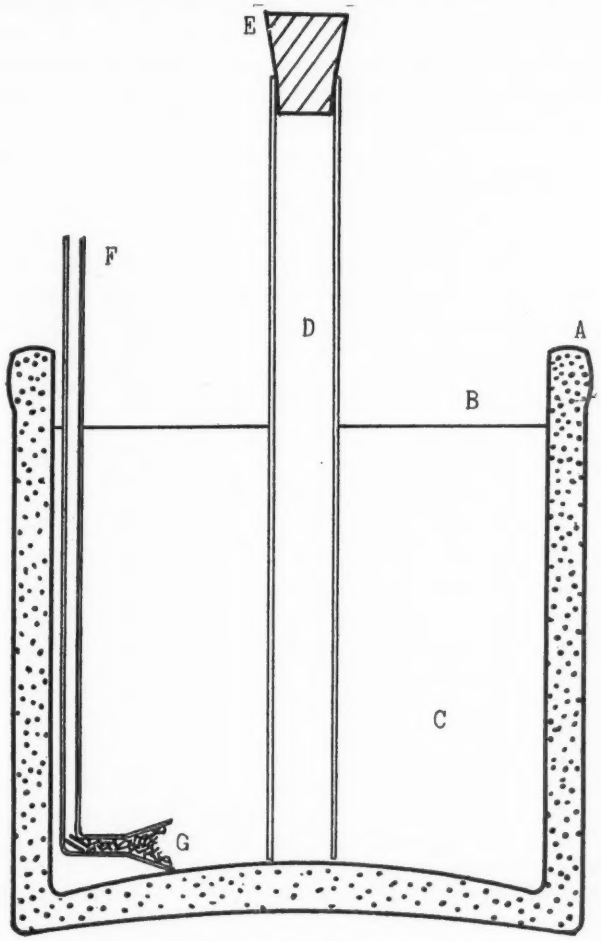

FIG. 1. DIAGRAM SHOWING CROSS-SECTION OF CULTURE POT AND TUBES FOR RENEWING AND FOR WITHDRAWING SOLUTIONS

A, glazed earthenware pot; *B*, wax seal; *C*, sand mass; *D*, supply tube with cork stopper *E*; *F*, outlet tube for withdrawing solutions by suction; *G*, glass wool.

A small glass tube was inserted from this larger opening into the bend of the tube and a tuft of glass wool wedged in next to the small piece of tubing. Suction was applied at the upper end of this tube whenever the solution was drawn off. Another glass tube, 2 cm. in diameter, ran through the center of the sand mass to the bottom of the pot. Into this tube fresh solutions were poured. These tubes are diagrammatically represented in figure 1. Solutions added at the bottom and allowed to rise through the sand are likely to disturb the plant roots less than those added at the top that flow rapidly down over

TABLE 1

Molecular proportions and partial volume-molecular concentrations of monobasic potassium phosphate, calcium nitrate and magnesium sulfate required to produce 21 solutions, each having a calculated osmotic pressure of 1.00 atmosphere at 25°C.

CULTURE NUMBER	SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME-MOLECULAR CONCENTRATIONS		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1	IR_1S_1	1	1	6	0.0027	0.0027	0.0161
2	S_2	1	2	5	0.0025	0.0049	0.0123
3	S_3	1	3	4	0.0024	0.0071	0.0094
4	S_4	1	4	3	0.0022	0.0089	0.0067
5	S_5	1	5	2	0.0022	0.0108	0.0043
6	S_6	1	6	1	0.0020	0.0122	0.0020
7	R_2S_1	2	1	5	0.0053	0.0027	0.0132
8	S_2	2	2	4	0.0049	0.0049	0.0099
9	S_3	2	3	3	0.0047	0.0071	0.0071
10	S_4	2	4	2	0.0045	0.0090	0.0045
11	S_5	2	5	1	0.0041	0.0104	0.0021
12	R_3S_1	3	1	4	0.0076	0.0025	0.0101
13	S_2	3	2	3	0.0072	0.0048	0.0072
14	S_3	3	3	2	0.0068	0.0068	0.0045
15	S_4	3	4	1	0.0065	0.0086	0.0021
16	R_4S_1	4	1	3	0.0099	0.0025	0.0074
17	S_2	4	2	2	0.0094	0.0047	0.0047
18	S_3	4	3	1	0.0090	0.0068	0.0022
19	R_5S_1	5	1	2	0.0123	0.0024	0.0049
20	S_2	5	2	1	0.0118	0.0047	0.0023
21	R_6S_1	6	1	1	0.0145	0.0024	0.0024

the roots. At the time the solutions were renewed distilled water was first added to bring the total weight up to the original total weight. Enough solution (500 cc.) was then added to bring the level of the water-table to the surface of the sand. After standing about four minutes suction was applied to the smaller tube and the culture brought back to its original weight.

The solutions used were those designated as type I by the Committee on Salt Requirements of Representative Agricultural Plants.[2] These solutions

[2] See specially prepared plans on the salt requirements of representative agricultural plants to be obtained from the chairman of Committee on Salt Requirements of Representative Agricultural Plants, Laboratory of Plant Physiology, Johns Hopkins University, Baltimore, Maryland.

were composed of 21 combinations of the three salts; monobasic potassium phosphate (KH_2PO_4), calcium nitrate ($Ca(NO_3)_2$) and magnesium sulfate ($MgSO_4$) when the partial osmotic pressure of each varied by equal increments of one-eighth of the total osmotic pressure. The initial total osmotic pressure of each solution was approximately 1.00 atmosphere. The molecular proportion and the partial volume-molecular concentration of each salt in each of the 21 solutions is given in table 1. An additional culture treated with distilled water was introduced into the series for comparison. No ferric phosphate was added to these cultures as there was a sufficient amount of iron in the sand for plant use. With but few exceptions, solutions were renewed twice a week over a period of 8 weeks. Where these exceptions occurred distilled water was added to bring the cultures back to their original weights.

Data on transpiration, evaporation and temperature were obtained at the time the solutions were renewed. At the conclusion of the experiment, data on the relative vigor and appearance of the plants, stem height, green weight of the plants and of the tubers and dry weight of the plants were obtained. Only such data are here presented as bear on the problem of selecting the best proportions of these three salts for good growth of the potato plant and its tubers.

Presentation of results

There was considerable variation in the general vigor or health of plants in the same cultures. This variation was probably due in part to the fact that sprouts of three different sizes were planted in each pot and to the use of home-grown seed of this particular variety which is not nearly so uniform as that grown at higher altitudes or farther north. General vigor or health of individual plants can not very well be measured quantitatively, so a method of scoring as described by Free (2) was used to indicate this quality of the plants. Each plant was compared only with the plants of its particular group, as for example, all plants in group *A* were compared with each other. An illustration of the variation in behavior of sprouts of the three sizes when grown in the same medium is shown in culture 5. Two plants (*A* and *C*) of this culture have the highest numerical score, but plant *B* has a score next to the lowest. These data, with those showing the approximate percentages of yellow leaf area, are presented in table 2. The greatest percentage of yellow leaf area occurs in culture 22 where nothing but distilled water was used.

The total green weight of the sprouts just before planting and the total green weight of plants (tops and roots without new tubers) at the time of harvest are given per culture in table 3 in grams and in numbers relative to the average total weight of sprouts. These sprouts when planted were not as uniform as was desired, but an examination of the ratios of final to original green weight shows the effect of various cultural treatments to be greater than that expected from individual variation alone. The final green weight

of plants is greatest for culture 15 while that of culture 14 is second. Cultures 5, 8 and 9 also are very good. The greatest ratio value occurs for culture 20, but this is due to the low initial weight of sprouts. The green weights of new tubers produced by the plants of this series are given in the last two columns of table 3. The seven cultures producing the greatest weight of tubers are 5, 14, 4, 6, 15, 13 and 8. The average weight per culture of the entire series is 32.3 gm.

TABLE 2

Relative vigor and percentage of yellow leaf area in potato plants of series I at the time of harvest

CULTURE NUMBER	NUMERICAL SCORE* OF GENERAL VIGOR			APPROXIMATE PERCENTAGE OF YELLOW LEAF AREA†		
	A	*B*	*C*	*A*	*B*	*C*
1	6	4[b]	10[b]	30	2	4
2	7	15	13[bb]	4	2	1
3	10	3	10	12	5	0
4	18	12	17	6	8	0
5	21	1	21	1	25	0
6	16	9	13[b]	2	70	2
7	11	2	3	25	5	10
8	17	7	19	15	10	0
9	20	16	18	2	2	0
10	8	16[b]	6	7	0	1
11	12	14	10[b]	1	3	0
12	3[a]	7[b]	9	10	10	3
13	18[b]	12[b]	8	7	4	2
14	15	21	14	2	2	6
15	14	19	20	1	10	10
16	1	6	4[b]	25	18	16
17	3[a]	11	11	1	12	0
18	11[b]	20	13	1	10	5
19	5	6	4	8	8	2
20	7[b]	18	1	7	0	0
21	2	4	2	4	40	0
22	0	0	0	100	100	80

* In several cases plants have the same numerical score. Where such cases occur the better plant is indicated by the superscript (b) while those that are alike have the superscript (a).

† These estimates were made by Prof. J. B. S. Norton.

The data of table 3 are perhaps more clearly presented as graphs in figure 2. The culture numbers are given in order along the abscissa. The first heavy line over the first six culture numbers represents the first row of cultures in the triangle diagram described in various publications[3] and illustrated in figures 4 and 5. The second heavy line over cultures 7 to 11, inclusive, is the second row of cultures in the triangle diagram. The third heavy line is the third row of cultures, etc., culture 21 being the apex of the triangle. All

[3] For descriptions of triangular diagrams and their application to plant nutrition work see Hibbard (3), McCall (7), Shive (11), Schreiner and Skinner (9, 10) and Tottingham (14).

graphs are plotted from the relative numbers in the table. In the lower half of the figure the full horizontal line represents the average green weight of sprouts at the time of planting, the broken line represents the green weight of sprouts of individual cultures and the full irregular line represents the green weight of the plants (tops and roots without tubers) at the time of harvest. Variation in weight of the sprouts is shown by the departure of

TABLE 3

Green weight of potato sprouts when planted and of plants and tubers at harvest with ratio of original to final green weight of plants given per culture of series I

CULTURE NUMBER	TOTAL GREEN WEIGHT OF PLANT				RATIO OF FINAL TO ORIGINAL GREEN WEIGHT	TOTAL GREEN WEIGHT OF TUBERS	
	Original		Final				
	Actual	Relative to average 18.1 gm.	Actual	Relative to average 18.1 gm.		Actual	Relative to average 32.3 gm.
	gm.		*gm.*			*gm.*	
1	17	0.94	33.1	1.83	1.95	25.7	0.80
2	16	0.88	33.5	1.85	2.09	25.0	0.77
3	17	0.94	38.2	2.11	2.25	27.0	0.84
4	21	1.16	49.9	2.76	2.38	39.9	1.24
5	21	1.16	52.6	2.91	2.50	43.6	1.35
6	22	1.22	48.1	2.66	2.19	39.9	1.24
7	26	1.44	42.2	2.33	1.62	29.2	0.90
8	25	1.38	53.9	2.98	2.16	37.4	1.16
9	20	1.10	53.7	2.97	2.68	29.3	0.91
10	16	0.88	44.3	2.45	2.77	31.3	0.97
11	18	0.99	45.4	2.51	2.52	35.4	1.10
12	16	0.88	32.1	1.77	2.01	23.2	0.72
13	18	0.99	50.5	2.79	2.81	38.8	1.20
14	18	0.99	56.7	3.13	3.15	42.2	1.31
15	20	1.10	57.4	3.17	2.87	39.9	1.24
16	17	0.94	45.6	2.52	2.68	28.5	0.88
17	16	0.88	44.3	2.45	2.77	26.7	0.83
18	16	0.88	50.9	2.81	3.18	33.3	1.03
19	16	0.88	38.3	2.12	2.39	32.0	0.99
20	12	0.66	38.6	2.13	3.22	35.4	1.09
21	15	0.83	35.8	1.98	2.39	27.8	0.86
22	17	0.94	22.8	1.26	1.34	18.1	0.56

the broken line from the horizontal line. The production of tops and roots is greatest in culture 15 with culture 14 a close second. In general as the position of a culture is moved from left to right along the heavy lines representing the culture rows the final green weight is increased. This suggests that either an increase in the proportion of calcium nitrate or a decrease in the proportion of magnesium sulfate increases the green weight production. There are, however, indications that a too great proportion of calcium nitrate or a too small proportion of magnesium sulfate decreases green weight pro-

duction. The question as to whether the calcium nitrate or the magnesium sulfate is the controlling salt will be discussed later.

In the upper portion of figure 2 graphs of tuber production (broken line) and of the ratio of final green weight to original green weight of the plants (full line) are shown. There is a striking similarity between the graph of tuber production and that of final green weight production. With but few exceptions the shapes and slopes of the curves are identical. The ratio graph has the appearance of a series of rises with each succeeding rise higher than the one immediately preceding. The highest point (culture 20) as stated

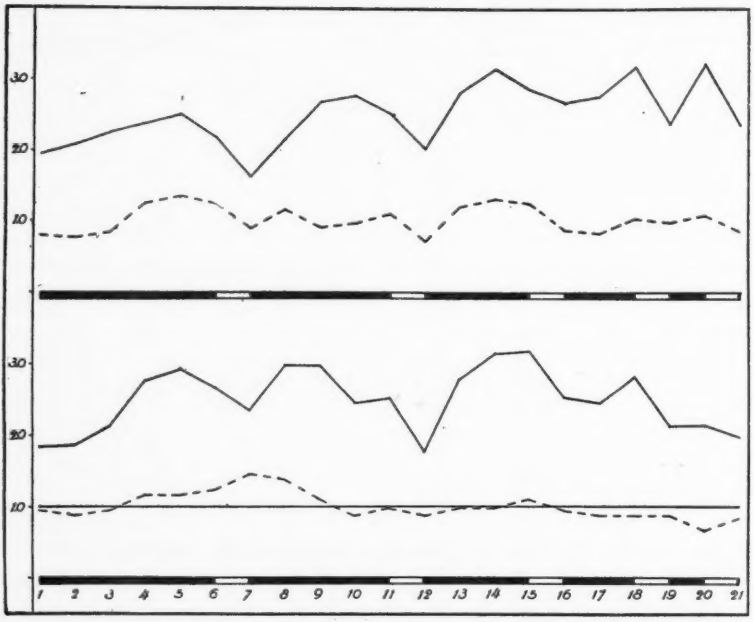

FIG. 2. Graphs of series I showing average green weight of sprouts (horizontal line), green weights of sprouts per culture (broken line) and green weight of plants per culture at harvest (full line) in lower part of figure; green weight of tubers (broken line) and ratio of final green weight of plants to green weights of sprouts (full line) for each culture in upper part of figure.

before is largely due to the low weight of these sprouts at the time of planting. The weight of sprouts in culture 20 was farthest below the average for the series while that of culture 7 was highest above the average, which latter fact accounts for the very low drop in the upper graph for culture 7.

The stem height of each plant (distance from surface of wax seal to base of terminal bud) is recorded in table 4. The variation in stem height between plants of the same culture is considerable in several cases, but when the

averages are compared culture 8 is found to contain the tallest plants and 22 the shortest. All the plants were more or less dwarfed. The transpirational water loss of each culture for the entire period is also given in this table, as well as the dry weights of the plants. Before obtaining the dry weights, the plants were first air-dried in the greenhouse and then placed in an electric vacuum oven at a temperature of 83°C. for 24 hours. The water require-

TABLE 4

Stem height of potato plants and transpirational water loss, dry weight of plants and water requirement per culture of series I

CULTURE NUMBER	STEM HEIGHT				TOTAL TRANSPIRATION	DRY WEIGHT OF TOPS AND ROOTS	WATER REQUIREMENT
	Plant a	Plant b	Plant c	Average			
	cm.	*cm.*	*cm.*	*cm.*	*gm.*	*gm.*	
1	7.0	1.0	2.0	3.3	1234	2.9	425
2	3.4	3.7	2.0	3.0	1109	2.8	396
3	6.4	2.2	2.4	3.7	1230	2.6	473
4	10.1	2.5	3.5	5.4	1616	4.0	404
5	11.5	1.2	3.3	5.3	1957	4.1	477
6	9.4	2.0	2.5	4.6	1730	3.7	468
7	5.0	2.1	3.0	3.4	1144	3.2	357
8	9.5	5.6	3.0	6.0	1437	4.5	319
9	8.5	2.4	5.0	5.3	1698	5.0	340
10	8.1	4.1	4.5	5.6	1203	4.2	287
11	10.3	3.8	3.6	5.9	1465	3.3	444
12	4.3	3.9	2.1	3.4	905	2.4	377
13	6.5	3.0	3.0	4.2	1544	3.5	441
14	6.4	6.6	2.5	5.2	1697	3.3	514
15	5.6	4.4	4.4	4.8	1523	4.7	324
16	3.5	1.4	3.3	2.7	890	2.8	318
17	3.3	2.0	3.0	2.8	934	3.6	259
18	6.5	2.5	2.5	3.8	1439	3.9	369
19	3.5	3.2	2.2	3.0	1030	2.4	429
20	3.5	4.4	1.6	3.2	1201	2.6	462
21	2.7	4.0	1.8	2.8	857	2.2	390
22	1.5	1.7	1.5	1.6	565	1.3	435
Average				4.0	1291		396

ments of these plants (transpiration per unit dry weight of plant) are given in the last column of the table. The mean water requirement for the entire series is 396 with a standard deviation of 67 ± 6.8.

SERIES II

Introductory

The culture pots used in series II had a capacity of 2 gallons each instead of 1 gallon as in series I. The amount of sand used was just twice that used in the first series, but the same moisture content (15 per cent based on the

weight of air-dry sand) was maintained. This increase in the size of the culture pot was made in order to give the roots and tubers ample room for growth and to maintain a more uniform moisture content. The amount of water lost by transpiration for a period of three or four days is relatively great for large plants. By increasing the total amount of solution and size of container the percentage of drying out is much less than would be the case where a smaller container is used. The percentage decrease of any one ion or molecule is also much less where greater amounts of solution are used. These points have been emphasized by Hoagland (4) and deserve more attention than has heretofore been given them.

Procedure and method of experimentation

The arrangement of tubes and plants in the pots was the same as that employed in series I. As has been stated, the amount of sand used was twice as great and hence the amount of solution was doubled in order to maintain the same moisture content as the cultures of series I. This weighed amount of air-dry sand was placed in each of 22 pots and then carefully washed with tap water and later with distilled water. The sand was then flooded with the solution to be used in that particular pot.

The seed ends of 144 Western-Maryland-grown Irish Cobbler potatoes were planted in a bed of sawdust on February 12, 1920. On March 18, sprouts similar in size and appearance with four or five well developed leaves were detached from their tubers and washed in tap water and then in distilled water. These sprouts were planted in sand, three to a container, and the cultures treated in a manner similar to that of series I. The check culture, number 22, was treated somewhat differently, however. A seed piece with three sprouts of the size used in the other cultures was carefully selected. This seed piece with its three sprouts was planted in the sand and treated in the same manner as the other 21 cultures with the exception that distilled water was used instead of a nutrient solution.

The weight of the cultures (about 14 or 15 kilos each) made it impracticable to use the rotating table employed in series I. A stone top table in the central part of the greenhouse was therefore used to support these cultures. To facilitate flooding the sand with solutions at the time the solutions were renewed, a liter flask containing the proper solution was placed on a small shelf in front of the culture and connected by a siphon to the central glass tube in the pot. The bottom of the flask was raised slightly higher than the surface of the wax seal. Such an arrangement of siphons made it possible to flood all the cultures at the same time. The cultures were permitted to remain in this saturated condition for several minutes before suction was applied and the total weight of each reduced to its original value.

Presentation of results

The three plants of each culture in the second series were much more uniform in appearance than those of the first series. No special scoring of relative health or vigor was made, but measurements similar in character to those of the earlier series were recorded during the experiment and at the time of harvest (May 6) and similar tables and graphs constructed.

TABLE 5

Green weight of potato sprouts when planted and of plants and tubers at harvest with ratio of original to final green weight of plants given per culture of series II

CULTURE NUMBER	TOTAL GREEN WEIGHT OF PLANT				RATIO OF FINAL TO ORIGINAL GREEN WEIGHT	TOTAL GREEN WEIGHT OF TUBERS	
	Original		Final				
	Actual	Relative to average 25.4 gm.	Actual	Relative to average 25.4 gm.		Actual	Relative to average 81.8 gm.
	gm.		*gm.*			*gm.*	
1	25.0	0.98	54.8	2.16	2.19	48.9	0.60
2	27.6	1.09	77.9	3.07	2.82	65.5	0.80
3	25.4	1.00	94.6	3.72	3.72	87.2	1.07
4	24.8	0.98	90.4	3.56	3.65	78.9	0.96
5	26.7	1.05	107.4	4.23	4.02	91.4	1.12
6	25.0	0.98	93.3	3.67	3.73	81.8	1.00
7	24.7	0.97	52.0	2.05	2.11	48.0	0.59
8	28.1	1.11	83.4	3.28	2.97	77.2	0.94
9	25.1	0.99	102.5	4.04	4.08	91.7	1.12
10	25.3	1.00	120.3	4.74	4.76	132.5	1.62
11	23.1	0.91	109.0	4.29	4.72	105.8	1.29
12	28.4	1.12	53.6	2.11	1.89	49.0	0.60
13	25.5	1.00	98.8	3.89	3.87	91.8	1.12
14	24.3	0.96	128.3	5.05	5.28	113.0	1.38
15	27.4	1.08	160.0	6.30	5.84	127.0	1.55
16	26.5	1.04	66.2	2.61	2.50	51.2	0.63
17	23.1	0.91	85.0	3.35	3.68	89.4	1.09
18	25.3	1.00	124.6	4.91	4.93	130.3	1.59
19	24.4	0.96	50.4	1.98	2.07	39.6	0.48
20	23.3	0.92	81.3	3.20	3.49	80.0	0.98
21	23.6	0.93	49.0	1.93	2.08	37.6	0.46
22	122.5*†		121.4*			49.4†	0.60

* Includes weight of old tuber. These weights are not included in the average weight.
† Weight not included in average weight.

Table 5 gives data of the total green weight of sprouts per culture when planted and that of the plants at the time of harvest, 7 weeks later. The ratio of final to original green weight and the green weight of tubers produced per culture also are presented in this table.

These data are represented graphically in figure 3, which is constructed from the relative numbers in table 5. The deviation in the weight of sprouts

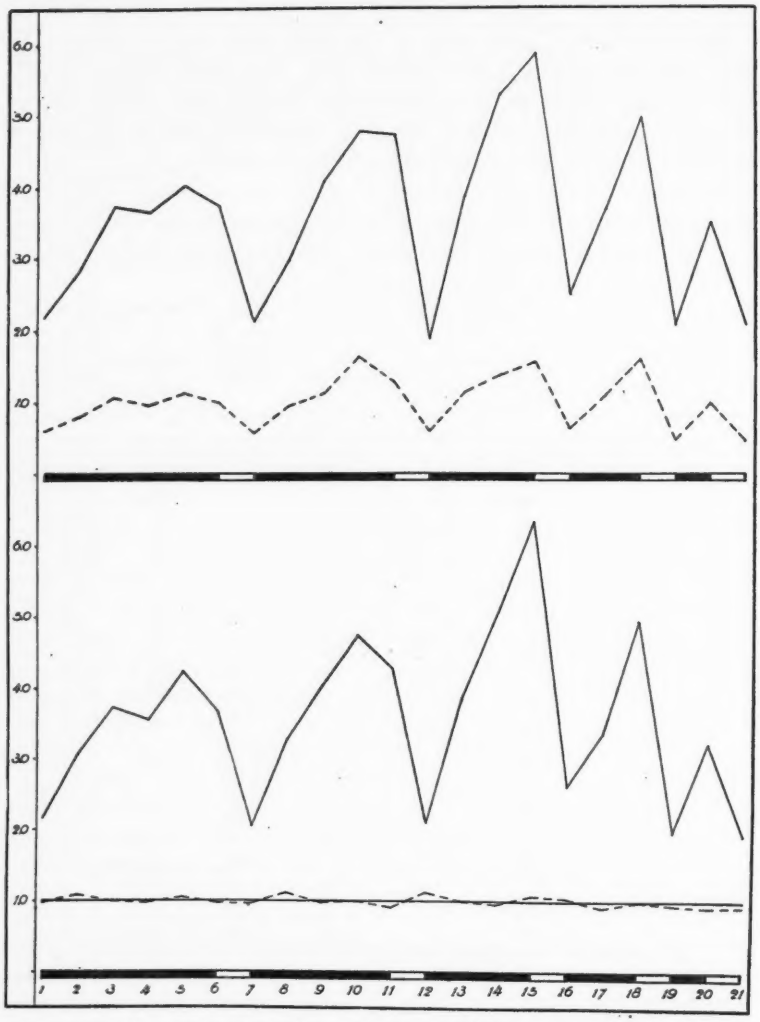

FIG. 3. Graphs of series II showing average green weights of sprouts (horizontal line), green weight of sprouts per culture (broken line) and green weight of plants per culture at harvest (full line), in lower part of figure; green weight of tubers (broken line) and ratio of final green weight of plants to green weights of sprout (full line) for each culture, in upper part of figure.

(lower broken line) from the average of the series (full horizontal line) is seen to be very small while differences in the final weight of plants (lower full line) are very marked. Culture 15 shows the greatest green weight. The series of peaks in the graph are located above the culture numbers near the right end of the heavy lines representing the culture rows of the triangle. The cultures having low green weight values are at the left end of the culture-row lines in every case. The graph of tuber production (upper broken line) is very similar to that of the final green weight. The maximum production

TABLE 6

Stem height of potato plants and transpirational water loss, dry weight of plants and water requirement per culture of series II

CULTURE NUMBER	STEM HEIGHT				TOTAL TRANSPIRATION	DRY WEIGHT OF TOPS AND ROOTS	WATER REQUIREMENT
	Plant a	Plant b	Plant c	Average			
	cm.	*cm.*	*cm.*	*cm.*	*gm.*	*gm.*	
1	6.8	7.0	5.5	6.4	2175	5.2	418
2	11.5	12.0	11.4	11.6	3127	8.1	386
3	12.9	11.4	14.0	12.8	4018	10.4	386
4	14.8	13.5	12.1	13.5	3590	12.1	297
5	11.0	15.5	17.0	14.5	4139	9.8	422
6	11.8	14.0	16.9	14.2	3813	9.3	410
7	4.5	5.3	5.5	5.1	1970	5.0	394
8	9.6	10.5	10.0	10.0	3287	7.7	427
9	13.8	15.7	14.5	14.7	4120	10.0	412
10	15.3	20.3	19.7	18.4	5138	14.2	362
11	16.8	15.9	17.5	16.7	4540	11.1	409
12	4.5	5.5	5.0	5.0	2272	5.2	437
13	10.7	10.7	9.5	10.3	3974	9.5	418
14	16.4	18.7	17.8	17.6	5066	13.1	387
15	12.7	22.8	20.0	18.5	5643	17.5	322
16	6.6	5.8	5.5	6.0	2303	5.7	404
17	11.3	12.2	7.5	10.3	3456	7.8	443
18	16.0	19.7	14.7	16.8	5163	11.3	457
19	4.4	5.9	8.0	6.1	1812	4.0	453
20	7.4	10.0	10.1	9.2	3146	7.1	443
21	5.0	4.0	6.7	5.2	1762	4.1	430
22	5.1	6.4	3.1	4.9	1122	2.3	488
Average				11.3	3438		409

occurs in culture 10 rather than in culture 15, however. Very little difference is noticed between cultures 10, 15 and 18 in tuber yield. The ratio graph (upper full line) showing the gain in green weight of the plants has its maximum at culture 15 and its minimum at 12. The two preceding and the two following maxima decrease in value the farther they are removed from this central maximum. With the shortening of the heavy base lines representing culture rows the proportion of potassium phosphate increases. The series of maxima in the upper graph indicates that with an increase of potassium

phosphate up to the third row, better plants are produced, but beyond that row decreased growth is noticed. The graph also shows that an increase of calcium nitrate up to the fourth or fifth culture of each row, where the rows are that long, produces increased growth. This is indicated by the rise in the curve above the heavy base lines representing culture rows.

Measurements of stem height per plant and the average for each culture are presented in table 6. Transpirational water loss for the entire period, dry weight of plants and the water requirement also are given for each culture in this table. The maximum average height value occurs for culture 15 and the minimum value for culture 22. These same cultures have the maximum and minimum transpirational values, respectively. The dry weight value for culture 15 is almost eight times that of the minimum, 2.3 gm. for culture 22. Since culture 22 received no fertilizer treatment and its plants were attached to their seed piece it is not comparable with any of the 21 cultures of the triangle. The maximum average height value of culture 15 is almost four times that of culture 12 of the triangle and its dry weight value is more than four times that of culture 19, the two cultures whose respective values are lowest. No such variations between cultures occur for the water requirement. The mean water requirement for this series is 409 with a standard deviation of 42 ± 4.3.

CONCLUSIONS

Introductory

Potato plants of corresponding cultures of the two series show great similarity in their reaction to the same salt proportions of the 21 different treatments. There are minor variations, but these are to be expected where a plant of considerable individual variation is employed, especially when two lots of seed are used. There is also the possibility of seasonal conditions bringing about different kinds of reactions in plants at different stages of development. This may account for the variation between plants of the same cultures in series I for the sprouts in each culture of this series were of three different sizes when planted. The seasonal differences, together with the use of home-grown seed, no doubt account for the smaller plants produced in series I. This series was grown at a time of year when light conditions were at a minimum. In spite of minor differences between the two series the results can be legitimately averaged and general conclusions deduced therefrom.

The green weight of the plants at the time of harvest, the dry weight of these same plants, the green weight of tubers and the water requirement for corresponding cultures of these two series have been averaged and are presented in table 7. All the average weights are given in grams per culture and as numbers relative to the average of each respective kind of measurement. The water requirement values are the averages of those given in tables 4 and 5.

TABLE 7

Average green and dry weights of potato plants, average green weight of tubers and average water requirement for corresponding cultures of series I and II

CULTURE NUMBER	WEIGHT OF PLANTS (TOPS AND ROOTS)				GREEN WEIGHT OF TUBERS		WATER REQUIREMENT
	Green weight		Dry weight				
	Actual	Relative to average 67.4 gm.	Actual	Relative to average 6.2 gm.	Actual	Relative to average 57.4 gm.	
	gm.		*gm.*		*gm.*		
1	44.0	0.65	4.1	0.66	37.3	0.65	422
2	55.7	0.83	5.5	0.89	45.3	0.79	391
3	66.4	0.99	6.5	1.05	57.1	0.99	430
4	70.2	1.04	8.1	1.31	59.4	1.03	351
5	80.0	1.19	7.0	1.13	67.5	1.18	450
6	70.7	1.05	6.5	1.05	60.9	1.06	439
7	47.1	0.70	4.1	0.66	38.6	0.67	376
8	68.7	1.02	6.1	0.98	57.3	1.00	373
9	78.1	1.16	7.5	1.21	60.5	1.05	376
10	82.3	1.22	9.2	1.48	81.9	1.43	325
11	77.2	1.15	7.2	1.16	70.6	1.23	427
12	42.9	0.64	3.8	0.61	36.1	0.63	407
13	74.7	1.11	6.5	1.05	65.3	1.14	430
14	92.5	1.37	8.2	1.32	77.6	1.35	451
15	108.7	1.61	11.1	1.79	83.5	1.45	323
16	55.9	0.83	4.3	0.69	39.9	0.70	361
17	64.7	0.96	5.7	0.92	58.1	1.01	351
18	87.8	1.30	7.6	1.23	81.8	1.43	413
19	44.4	0.66	3.2	0.52	35.8	0.62	441
20	60.0	0.89	4.9	0.79	57.7	1.01	453
21	42.4	0.63	3.2	0.52	32.7	0.57	410

Plant production

Green weight. The maximum green weight is 108.7 gm. for culture 15. This represents the weight of three plants. The minimum weight is 42.4 gm. for culture 21. The maximum is approximately two and one-half times greater than the minimum. The average weight for the entire series is 67.4 gm. The weights of the individual cultures are expressed in the third column of the table as numbers relative to this average, for the purpose of simplifying comparisons between cultures. Eleven cultures are above the average, or 1.00, and the remaining ten below. Cultures 3 and 17 are almost as good as the average, however.

The green weights of these plants are represented diagrammatically in figure 4. In general form the figure is similar to those ordinarily used to represent a triangle of cultures. Instead of merely representing the high and low areas, this figure has been made to resemble a contour map. The seven cultures of lowest values are shown in the "swamp land" while the other

fourteen are placed at different "elevations" by contour lines. These contour lines are drawn for every 10 gm. of green weight above 60 gm. The cultures are numbered in regular order in the triangle. Culture 17, for example, lies between the contour lines 60 and 70. The plants of this culture have a total green weight between 60 and 70 gm. Cultures 3, 8 and 20 lie between the same contour lines. Culture 15 has the highest value and is encircled by a

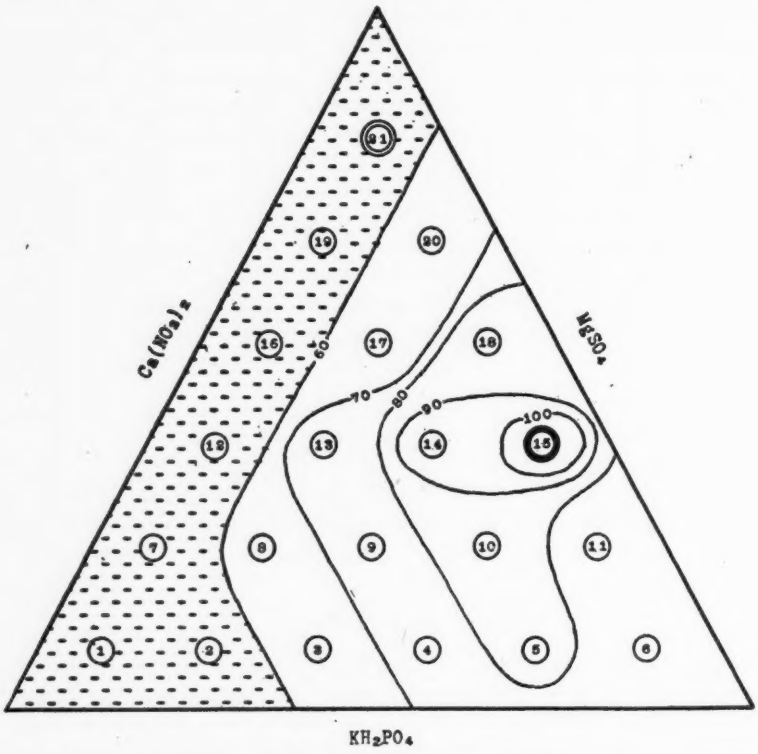

FIG. 4. DIAGRAM SHOWING THE APPROXIMATE GREEN WEIGHTS OF POTATO PLANTS (TOPS AND ROOTS) PER CULTURE

The seven lowest-yielding cultures are within the shaded area below the 60-gm. contour line; the culture giving the highest yield is marked by the heavy circle, the lowest by the double circle.

heavy ring. Culture 21 has the lowest value and is encircled by a double ring. Such a diagram enables one to see at a glance the relation of various cultures to each other with respect to their comparative yields and to their position in the triangle, thus showing their relation to various proportions of the three salts.

When the green weight is used as a criterion, greatest growth is obtained from cultures high in calcium nitrate, low in magnesium sulfate and with a medium amount of potassium phosphate. The question previously raised as to whether high calcium nitrate or low magnesium sulfate is responsible for this better growth can be answered in part. Further experimentation, however, where the ions and elements of these salts are interchanged and are used in other combinations, must be carried out before the controlling ions and elements of these salts can be definitely known in their true relation to the growth and development of the plant. An examination of plate I showing the plants of series II will bring out certain facts. Cultures lying along the calcium nitrate side of the triangle, numbers 1, 7, 12, 16, 19 and 21, are very much alike in appearance and size. An examination of the figures in tables 5 and 6 as well as the average values in table 7 will bear out the same fact. All of these cultures contain one part of calcium nitrate while they vary in their content of magnesium sulfate from 1 to 6 parts. It can not be said that plants of culture 1 are six times as large or six times as small as those of culture 21. This would no doubt be the case if magnesium sulfate were the salt most influential in their growth. Furthermore, culture 21 contains six times the amount of potassium phosphate as culture 1, but there is not six times as much difference in growth between the two. With an increase of calcium nitrate, however, there is an increase in vigor, weight and height of the plants. The rise in the graphs along the culture rows as brought out in connection with figures 2 and 3 also emphasizes this.

Dry weight. The figures in the second double column of table 7 giving the dry weights of plants show variations between cultures similar to those of the green weights. In general, there is little difference between the relative numbers of green and dry weight for plants of corresponding cultures.

Tuber production

The economic importance of the potato is due to its tuber and the ultimate aim of culture work is the working out of a properly balanced fertilizer that will bring about the greatest yield of tubers. With the nutrient elements contained in the three salts here used considerable differences in amounts of new tubers produced were obtained in the various cultures. The average yield of the two series is given in grams and in numbers relative to the average yield in the third double column of table 7. The greatest yield (83.5 gm.) is shown for culture 15 while cultures 10 and 18 give almost as good yields. The minimum yield of 32.7 gm. is found in culture 21. The maximum is about 2.6 times as great as the minimum. This is practically the same ratio that exists between the maximum and minimum green weights of the plants on which these tubers grew.

The relative numbers make comparisons between cultures and between these three kinds of measurements (green and dry weights of plants and green

weight of tubers) easy. There is a striking similarity between all these relative values. The average green weight of the plants is about 17 per cent heavier than the average green weight of the tubers. This relation holds good for individual cultures where the relative values are alike or nearly alike, which is the case in the majority of cultures.

The green weight of tubers of the 21 cultures is shown in figure 5 which is constructed similarly to figure 4. The seven cultures of lowest values lie

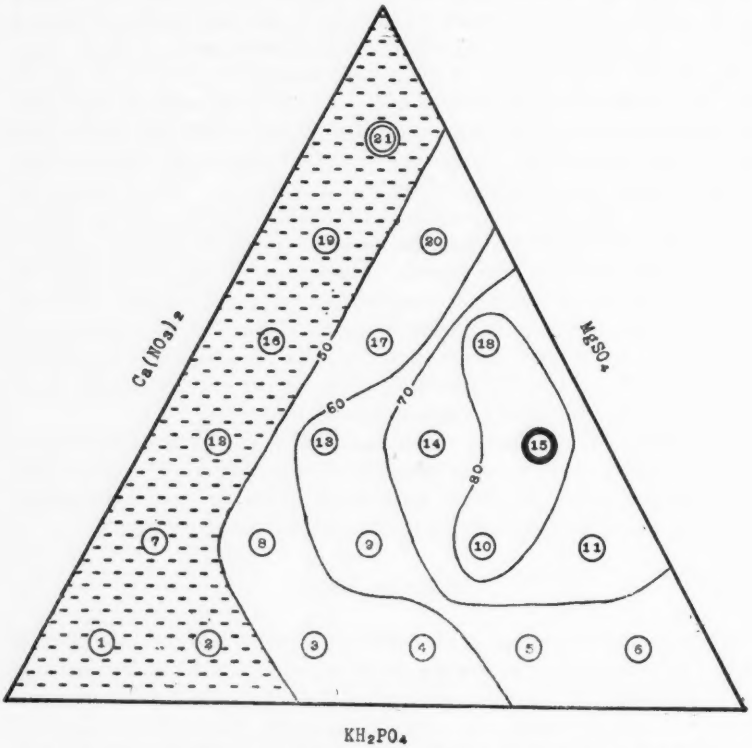

FIG. 5. DIAGRAM SHOWING THE APPROXIMATE YIELDS OF TUBERS PER CULTURE

The seven lowest-yielding cultures are within the shaded area below the 50-gm. contour line; the culture giving the highest yield is marked by the heavy circle, the lowest by the double circle.

in the "swamp land" while the contour lines are drawn for every 10 gm. of "elevation" above 50 gm. The highest culture (number 15) is encircled by a heavy ring while the lowest (number 21) is within the double ring. These two cultures are the same high and low ones of figure 4. There are minor differences in the shape of the contour lines, but in general there is marked

similarity between these two figures. Apparently the same proportions of salts that bring about good growth of plants (tops and roots) bring about good growth of tubers.

Height

The average height of plants in series I is 4.0 cm. while that in series II is 11.3 cm., or almost triple that of series I. This difference is probably due to seasonal differences of the two series.

Water requirement

The average water requirements of the cultures in the two series as given in table 7 show a maximum value of 453 for culture 20 and a minimum value of 323 for culture 15. The maximum value is approximately 1.4 that of the minimum, or there is a variation of about 40 per cent between maximum and minimum. This is a small variation when those of the other data in the table are considered. It also happens that culture 15 has the lowest water requirement and the highest green weight values of plants and tubers, but when the average water requirement values are arranged in descending order and plotted with the green weights of plants and tubers, culture for culture, there is apparently no relation seen. There seems to be a tendency for the water requirement value to remain constant under the various climatic conditions and in the various cultures of these experiments.

SUMMARY

The results obtained from two series of experiments dealing with the nutrient requirements of the Irish Cobbler potato plant are presented in this paper. Potato sprouts separated from their seed pieces were grown in sand cultures and treated with solutions designated as type I. This series of solutions consisted of 21 different salt proportions of monobasic potassium phosphate (KH_2PO_4), calcium nitrate ($Ca(NO_3)_2$) and magnesium sulfate ($MgSO_4$). The partial osmotic pressure of each varied by equal increments of one-eighth of the total osmotic pressure which was approximately 1.00 atmosphere.

Whether the green weight of plants (tops and roots together) or the green weight of new tubers produced is used as the criterion of growth, the best average values of corresponding cultures of the two series occurred for the cultures high in calcium nitrate and low in magnesium sulfate with a medium amount of potassium phosphate. Cultures giving the lowest yields were low in calcium nitrate. The average highest yielding culture was IR_3S_4 with the three salts in the following volume-molecular concentration: KH_2PO_4, 0.0065 *M.*; $Ca(NO_3)_2$, 0.0086 *M.*; $MgSO_4$, 0.0021 *M.* The average lowest yielding culture was IR_6S_1 with the following volume-molecular concentration: KH_2PO_4, 0.0145 *M.*; $Ca(NO_3)_2$, 0.0024 *M.*; $MgSO_4$, 0.0024 *M.*

The average water requirement for plants of the two series was 403. There was a marked tendency for individual cultures not to vary greatly from this value. There was apparently no relation between high yield and low water requirement and low yield and high water requirement.

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PLATE 1

CULTURES OF SERIES II ARRANGED IN THE FORM OF A TRIANGLE

Cultures on the left side are low in calcium nitrate, those on the right side low in magnesium sulfate and those on the base of the triangle low in monobasic potassium phosphate.

PLATE 1